VOLUME 19 NUMBER 2

NUTRITION REVIEWS

February 1961

Comments on the Current Food Situation, by Oris V. Wells..... 33

Clinical Nutrition

Dietary Management of Congestive Heart Failure, Hypertension and Angina Pectoris..... 35
Obesity, Growth, and Maturation in Childhood..... 36
Myocardial Infarction in Patients with Portal Cirrhosis..... 37
Magnesium Metabolism in Protein Malnutrition..... 38
Fat Mobilizing and Ketogenic Substances in Urine..... 39

Animal Nutrition

Multiple Origins of Muscular Dystrophy in Lambs..... 41

Experimental Nutrition

Exercise and Calcium Utilization..... 42
Starvation and Lactic Dehydrogenase..... 44
Effects of Diet and Age on Intestinal Flora in the Pig..... 47
Drugs Which Induce Ascorbic Acid Synthesis in Rats..... 48
Substances Influencing the Arginine Requirement..... 49
Vitamin E-Free Diets in Chicks..... 52
Fat Absorption and Utilization in Tumor-Bearing Rats..... 53
Physical Activity and Experimental Dietary Atherosclerosis..... 55
Hypertension and Salt Aversion..... 57
Mechanism of the Thiamine-Sparing Action of Dietary Penicillin in Rats..... 60
Essential Fatty Acid Activity of Trans Acids..... 62

Notes

Letter to the Editor..... 63
Methoxychlor—Not a Milk Residue Problem..... 63

Published Monthly at Mt. Royal and Guilford Aves., Baltimore, Md.

for

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

COPYRIGHT, 1961 NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass.

Assistant Editor

PRISCILLA GOREE, One Shattuck Street, Boston.

Associate Editors

GEORGE K. DAVIS, PH.D., Department of Animal Husbandry and Nutrition, University of Florida, Gainsville.
ALFRED E. HARPER, PH.D., Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.
ROBERT E. HODGES, M.D., Department of Internal Medicine, State University of Iowa, Iowa City.
STANLEY LEVEY, PH.D., Departments of Biochemistry and Surgery, College of Medicine, Western Reserve University, Cleveland.
JAMES F. MEAD, PH.D., Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles.
OLAF MICKELSEN, PH.D., Laboratory of Biochemistry and Nutrition, National Institute of Arthritis and Metabolic Diseases, Bethesda.
WILLIAM J. PYLES, M.D., St. Luke's Hospital, New York.
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston.
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester.
DONALD M. WATKIN, M.D., Organización Panamericana de la Salud, Mexico City.
ROBERT W. WISSLER, PH.D., M.D., Department of Pathology, University of Chicago.

Editorial Committee

WALLACE R. AYKROYD, M.D., University of London
CHARLES S. DAVIDSON, M.D., Boston City Hospital
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
IAGO GALDSTON, M.D., New York Academy of Medicine
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
BERWYN F. MATTISON, M.D., American Public Health Assn.
L. A. MAYNARD, PH.D., Cornell University
HAZEL STIEBELING, PH.D., Institute of Home Economics, U.S.D.A.
LEROY VORIS, PH.D., National Research Council
WARREN WEAVER, PH.D., Alfred P. Sloan Foundation
JOHN B. YOUMANS, M.D., Office of the Surgeon General, Dept. of the Army

Subscriptions should be sent to The Nutrition Foundation, Inc., 99 Park Ave., New York 16, N. Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries
Changes of address should be sent to The Nutrition Foundation.
Second-class postage paid at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

NUTRITION REVIEWS

VOL. 19 FEBRUARY 1961 No. 2

COMMENTS ON THE CURRENT FOOD SITUATION*

The current U. S. food situation is presented in terms of the food outlook for 1961, certain legislative questions referring to the special milk and school lunch programs, and the recent food resolution of the United Nations General Assembly and the Freedom-from-Hunger Program of the Food and Agricultural Organization.

Current Food Situation

U.S. per capita food consumption index for 1960 averaged about 103, basis 1947–49 = 100, or the same as for 1959. Actually, per capita food use has been remarkably stable for some time now. There are, of course, offsetting changes. For example, Americans consumed about four pounds less pork per capita last year and about four pounds more beef. The average level of per capita food consumption should continue at, or close to, the 1960 level throughout 1961. It is likely there will be more beef and poultry in 1961, perhaps somewhat fewer eggs (especially during the first six months).

Retail food prices for the fall of 1960 were about one per cent higher than for the corresponding month a year ago and it is estimated that they will remain at the current level throughout most of 1961.

Two continuing trends are the downtrend in egg consumption and the continuing shift from milkfat to nonfat milk solids, both of which have been underway since about 1940. Average per capita use of eggs in 1960 was about 325, which compares with 347 for 1959. Average use of eggs (farm basis) increased from 300 eggs per person during the 1930's to an average of 385 for the three years 1947–49. There was a time when it seemed that there might be something wrong with the U.S. statistics, but it increasingly looks as if there has been a real decline or downtrend in the average per capita use of eggs over the last several years—a decline which is difficult to explain unless more and more Americans are eating less and less for breakfast.

A second trend which continues is the shift down in average per capita use of milkfat, offset, at least until a year or two ago, by an increase in consumption of nonfat milk solids. The use of milkfat was down another one-half pound during 1960, bringing the total to 25 pounds per capita as compared with 32 pounds 20 years ago. Over the same two decades the average per capita use of nonfat milk solids has increased from 40 to 48 pounds. This shift in consumption is also accompanied by a decline in the average butterfat content of total milk production.

One other item of interest is the release of the results of a recent survey by the Pan American Coffee Bureau which estimates the amount of milk and cream used in the annual total consumption of coffee in the United States (estimated at 377 million cups a day or 137 billion cups a year). This survey indicates that more milk (or milk and cream expressed in terms of whole milk equivalent) goes into coffee than into ice cream and other frozen dairy products or almost as much as goes into cheese. This estimate comes as a surprise and, hopefully, more detail as to how the results of the survey were translated into the estimated total milk equivalent will be obtained.

Special Milk and School Lunch Programs

For several years now the U.S. has had a Special Milk Program under which government grants or payments of three or four cents per half pint of milk consumed under certain conditions are made to schools, as well as similar payments (two cents) to

*Adapted from a report to the Food and Nutrition Board, November 4, 1960.

summer camps. This is a popular program even though it does raise some questions when considered in relation to the National School Lunch Program. So far, it has been a Commodity Credit Corporation program based on the theory that it is better for school children to consume additional milk than for the Corporation to buy and store butter and dry skim milk. The current authorization for the program expires on June 30, 1961.

A subcommittee of the House Agriculture Committee has raised the question whether an extension of this program should be financed through the regular appropriations and has specifically asked that the United States Departmet of Agriculture and the various states consider three different issues in this connection: the method by which funds would be managed, assuming that the appropriation will be stabilized at some appropriate level; the role and responsibility of state and local governments; and the relationship of the Special Milk Program to the National School Lunch Program.

Meanwhile, the General Education Subcommittee of the House Committee on Education and Labor has also asked for help in studying alternative methods of apportioning school lunch funds among the states, incorporating the factors of participation and need. The current formula distributes funds among states on the basis of estimated school population and relative need (measured by average per capita income in each state), which means that the states with lower rates of participation have the higher reimbursement rates for school lunch, other things being equal. This request resulted from a hearing held by the Subcommittee in August, 1960.

Food Surpluses and Freedom from Hunger

Sometime ago the Governing Conference of the Food and Agriculture Organization authorized the Director-General to develop a "Freedom-from-Hunger" Campaign. This campaign has two main purposes: to increase interest in, and attention to, food and nutrition problems over the world; and to emphasize concerted action in mobilizing, on a voluntary basis, the efforts of the various agencies assisting underdeveloped countries to make progress on the food and nutrition front. The campaign is expected to extend over several years and will include provision for a World Food Congress in 1963.

This program was formally inaugurated on July 1, 1960; a considerable number of contributions have been made for the campaign and the various countries of the world are organizing Freedom-from-Hunger Committees. The matter was discussed with Director-General Sen during his last visit to the United States, and it is anticipated that a United States National Freedom-from-Hunger Committee, composed of well-known individuals and representatives of nongovernmental agencies, will be organized in the near future to assist and encourage United States participation in the campaign.

Meanwhile, there is a growing interest in the possible increased use of surplus foods in underdeveloped areas of the world. On October 27, 1960, the General Assembly of the United Nations adopted a "Provision-of-Food-Surpluses-to-Food-Deficient-People-Through-the-UN System" resolution which, among other things, endorses the Freedom-from-Hunger Campaign launched by the FAO and urges all members of the United Nations and of the specialized agencies to support this campaign in every appropriate way; appeals to members of the United Nations and the specialized agencies to take suitable measures to relieve the suffering of food-deficient people in other nations and assist them in their economic development and in their efforts toward a better life; and expresses the belief that international assistance in the establishment of national food reserves in food-deficient countries is one effective transitional means

to assist accelerated economic development in the less developed countries.

This resolution of course has a number of safeguarding clauses which stress that any action taken or contemplated should proceed in accordance with the FAO principles and guiding lines for the disposal of surplus commodities and should provide appropriate measures against dumping agricultural surpluses on international markets. The original sponsoring countries for the resolution were the United States, Canada, Haiti, Liberia, Pakistan, and Venezuela. Meanwhile, the FAO Council, simultaneously meeting in Rome, noted with gratification the UN resolution, authorized the Director-General of FAO to proceed with the action requested, and designated an Appropriate-Country Committee to consult with the Director-General.

ORIS V. WELLS
Administrator, Agricultural Marketing Service
U.S. Department of Agriculture
Washington, D. C.

DIETARY MANAGEMENT OF CONGESTIVE HEART FAILURE, HYPERTENSION AND ANGINA PECTORIS

The authors restricted salt, forced fluids, and gave supplements of potassium chloride with apparently excellent results in the management of several forms of cardiac disease.

Most clinicians regard dietary restriction of salt as an important facet in the management of congestive failure, but few restrict the salt intake of patients with hypertension or with angina pectoris. This attitude represents a compromise of many opinions. The most rigid salt restriction was advocated by W. Kempner (*Ann. Int. Med.* **31,** *821 (1949)*) who treated hypertensive patients with a diet composed of rice, fruit and fruit juices. However, many physicians allow salt to hypertensive patients unless congestive failure develops.

The question of fluid allowance in the diet has been settled by a compromise—between a high intake of water and water restriction with the result that most clinicians allow their cardiac patients to drink water at will.

In an interesting report from Mexico City, D. Sodi-Pallares (*Canad. Med. Assn. J.* **83,** *243 (1960)*) described his studies of the effects of salt restriction, a high intake of water, and extra potassium chloride upon the clinical course of three groups of cardiac patients. These included 83 patients with congestive heart failure due to various causes, 100 ambulatory patients with arterial hypertension, and 34 patients with severe angina pectoris. Apparently most of the patients were treated in the outpatient clinic and records were kept of their clinical condition including loss of edema, change of weight, improvement of symptoms, clearing of pulmonary rales, changes in the blood pressure, and tolerance to exercise. The regimen given to these patients was based upon the following therapeutic measures: (1) a high intake of water (between 2000 and 3000 cc. daily; (2) restriction of sodium chloride between 300 and 1500 mgs. per day; (3) a well-balanced diet providing all the necessary nutrients; and (4) added potassium chloride when needed to maintain the serum concentration of potassium above 4.2 mEq/l. In addition, the author gave oral diuretics to selected patients. A few were given adrenal cortical steroids substances in the hope of increasing diuresis or restoring their blood pressure to normal.

The duration of the studies varied from four to 210 days in the hypertensive patients, from four days to one year in the group of patients with angina and from five to 60 days in the patients with congestive heart failure.

The author reported that congestive heart failure responded remarkably well despite the fact that digitalis was given only to those patients who had tachycardia or an arrhythmia. The patients with hypertension sustained a mean decrease in systolic pressure of approximately 50 mm. of mercury and of diastolic blood pressure of approximately 20 mm. of mercury. The patients with angina were benefitted greatly. The author ascribed much of his apparent success to the use of a high intake of water which he felt might augment the diuresis. He also considered that careful attention to the prevention of hypokalemia was important. Restriction of sodium chloride and the use of diuretic agents were also considered significant factors. The author justified the restriction of salt in the group of patients with hypertension on the basis that it helped to maintain normal osmolarity of the blood. He quoted L. A. Sapirstein, W. L. Brandt and D. R. Drury (*Proc. Soc. Exp. Biol. Med.* **73,** *82 (1950)*) who proposed that one defect in hypertension might be an abnormal metabolism of water manifested by excessive losses through perspiration and a habitual disregard of thirst. The author suggested that much of the pain in angina pectoris is due to a degree of myocardial decompensation (subclinical congestive failure) which, if prevented, results in improvement of the clinical picture. He also suggested that a high concentration of intracellular sodium may potentiate the vasoconstrictor effects of catecholamines.

The author wisely admitted that his studies were of a preliminary nature and not fully controlled. This certainly is evident from the obvious lack of uniformity or standardization of the cases. The length of observation should have been similar in each case and preferably alternate cases should have been treated by more conventional methods.

While the author did make a plea for future appraisal of the principle of hydration of cardiac patients, he weakened his case by employing miscellaneous forms of therapy in addition to that under study and by providing incomplete data from their patients. Nevertheless, this experience seems sufficiently suggestive to warrant additional carefully controlled experiments to evaluate the effects of hydration and of prevention of hypokalemia upon the clinical course of these three conditions.

OBESITY, GROWTH, AND MATURATION IN CHILDHOOD

Fat children grow at a faster rate and mature earlier than thin children.

There is much evidence to support the contention that growth performance in man is a function of many factors, one of the most important being nutrition. Undernourished children do not grow at a normal rate. In recent years the converse of this question has been discussed: namely, do overnourished children grow at a super-normal rate?

Carefully compiled and extensive data have now been brought to bear on this question. S. M. Garn and J. A. Haskell (*Am. J. Dis. Child.* **99,** *746 (1960)*) report data on the relationship of the amount of subcutaneous fat in children to the rate of growth and maturation.

As an index of fatness, the width of the fat-plus-skin shadow was measured on routine chest roentgenograms at the level of the right tenth rib. A total of 259 clinically healthy white children ranging in age from 1½ to 17½ years were available for study. Measurements of stature, and of osseous maturation at the wrist were recorded, as well as age at menarche, and the age at which fusion of the proximal tibial epiphysis occurred. A few more boys than girls were included in the sample.

The authors then proceeded to construct statistical correlations between fat thickness and these attributes of developmental sta-

tus. At all ages between 4½ years and 11½ years in boys, and between 3½ years and 13½ years in girls, there was a significantly positive correlation between thoracic fat thickness and stature. The fattest individuals in the study were about a year ahead in stature as compared to the average child. Similarly, the fatter children exhibited a bone age about 0.4 year advanced over the normal.

Since these children were enrolled in a long-term longitudinal study, it was possible to attempt some long-range correlations. Fat thickness, measured at age 8.5 to 9.5 years, was shown to have a positive correlation with the age at which tibial fusion occurred in both boys and girls. (This event usually occurs at about 18 years of age.) Age at menarche could also be correlated with fat thickness at 8.5 years. Obesity thus appears to be associated with developmental acceleration in both sexes.

It should be noted here that the correlation coefficients were in the range of 0.3 to 0.4 for the males, and 0.3 to 0.6 for the females.

At both age extremes in the sample under study, the correlation between fat thickness and developmental status was not significantly greater than zero. Growth, of course, begins to slow down after puberty, so that the postulated influence of nutrition would naturally be restricted in its expression at that time. Furthermore, growth factors of a nonnutritional nature may well predominate in the young infant. Nutrition exerts its greatest influence in the middle years of childhood.

Other studies on the growth of obese children have been reviewed recently (*Nutrition Reviews* **14,** *172, 229 (1956)*), and it has been suggested that the documented secular changes in growth rate are in part due to better nutrition (*Ibid.* **15,** *193 (1957)*). The authors of the present study have reenforced the role of obesity by actually measuring the thickness of the subcutaneous tissue and using this as a criterion of the degree of obesity. Although obese children grow faster and develop more rapidly than thin children, it remains to be seen whether the obese child grows up to be a tall adult.

The results of this study support the hypothesis that growth and development are to a certain extent functions of nutritional status.

MYOCARDIAL INFARCTION IN PATIENTS WITH PORTAL CIRRHOSIS

Comparison of autopsy findings showed a decreased incidence of myocardial infarction in patients with portal cirrhosis as compared with other conditions.

Clinicians a few generations ago were as divided in their opinions about the relation of alcohol to atherosclerosis as they are today. R. C. Cabot (*J. Am. Med. Assn.* **43,** *774 (1904)*) discussed this problem and studied autopsy findings of a number of alcoholics. He concluded that he could find no evidence to support the concept that alcohol predisposed to arteriosclerosis. Indeed, a number of physicians felt that alcoholic beverages were beneficial to their elderly patients with angina pectoris (T. Leary, *New Eng. J. Med.* **205,** *231 (1931)*). Recently, medical opinion has been conservative or mildly opposed to the use of alcohol as a means of preventing atherosclerosis (O. M. Jankelson, J. J. Vitale, and D. M. Hegsted, *J. Clin. Nutr.* **7,** *23 (1959)*).

Still, sporadic reports have been made regarding the frequency of atherosclerosis in chronic alcoholic and cirrhotic patients; however, controlled studies have been lacking. W. L. Howell and W. C. Manion (*Am. Heart J.* **60,** *341 (1960)*) decided to study the coincidence of myocardial infarction and portal cirrhosis in the records of autopsies collected by the Armed Forces Institute of Pathology during the year 1957. The files contained over 17,000 records for that year, 90 per cent of which came from

former military personnel. Approximately 96 per cent of all cases were men, 84 per cent Caucasian, 15 per cent Negro. The mean age at death was 63 years.

The authors selected all cases having an anatomical diagnosis of portal cirrhosis, Laennec's cirrhosis, or post necrotic cirrhosis, a group of 639 cases whose average age at death was 58 years. They compared the incidence of the anatomic diagnosis of myocardial infarction in each group excluding such diagnoses as myocardial fibrosis, focal fibrosis, thrombotic occlusion of the coronary arteries, or arteriosclerotic occlusion of the coronaries. In a 5 per cent random sample of the total group of records, the incidence of myocardial infarction was 20.2 per cent. By contrast, the cirrhotic group had only 4.9 per cent incidence of infarction. Even in cirrhotic patients with diabetes mellitus the incidence of myocardial infarction was low (one out of 24 cases, average age 63 years). The incidence of recent infarction was compared and found to be 10 per cent in the control series and only 1.8 per cent in the cirrhotic patients. The authors stated that statistical analysis confirmed the significance of these data even though the mean ages were somewhat dissimilar.

If one accepts the fact that myocardial infarction is infrequent in cirrhotic patients, what are the possible factors which could explain this situation? Is the alcoholic patient genetically predisposed to one illness, yet protected from another, or does he spare himself the "stress" of his sober brethren by drinking? Is his diet so dissimilar from his fellow man that he avoids the atherogenic substances or does alcohol itself protect the vascular channels of the heart? Is the alcoholic patient deficient in certain vitamins which, in normal men, allow the process of atherogenesis to proceed unmolested? Perhaps his liver is so deranged that it cannot manufacture cholesterol or other substances important to the process of atherosclerosis.

The authors favored a different view, namely, that alterations in the balance between coagulation of blood and fibrinolysis might explain the phenomenon. They cited the work of E. W. Goodpasture (*Bull. Johns Hopkins Hosp.* **25,** *330 (1914)*; O. D. Ratnoff, *Bull. Johns Hopkins Hosp.* **84,** *29* (1949); H. C. Kwaan, A. J. S. McFadzean, and J. Cook, *Lancet* **I,** *132, 968 (1956)*) which indicates that fibrinolytic activity is increased in the blood of cirrhotic patients. It has been proposed (T. Astrup, *Lancet* **II,** *565 (1956)*) that formation of fibrin and lysis of this substance is a continuous process, and that an imbalance of this system may be responsible for deposition of fibrin upon the intimal surfaces of arteries, thus initiating the process known as atherosclerosis. The fact that hyperlipemia favors coagulation of blood (H. C. Roth, W. Rosenthal, and E. E. Mandel, *Proc. Soc. Exp. Biol. Med.* **101,** *516 (1950)* would allow this theory to coexist with that of the effect of dietary fats.

Whatever the mechanism may be, it would appear that the process of portal cirrhosis is accompanied by a sparing of the vascular intima, particularly of the coronary circulation.

MAGNESIUM METABOLISM IN PROTEIN MALNUTRITION

Low muscle magnesium content and decreased rates of magnesium excretion in urine were used as an indication of magnesium deficiency in children with kwashiorkor.

The relationship of various inorganic substances such as potassium, phosphorus and magnesium in muscle is quite constant. I. MacIntyre and D. Davidsson (*Biochem. J.* **70,** *456 (1958)*) have found that animals on magnesium-deficient diets develop a potassium deficiency correlating with the magnesium deficiency though of greater magnitude. A possible explanation may be that magnesium dependent systems in the mitochondria

are necessary to maintain the large intracellular potassium concentrations in comparison to low extracellular levels. A corollary of this relationship might be that if magnesium deficiency is not corrected, potassium deficiency cannot be corrected. With potassium deficiency there will be nitrogen deficits.

Since magnesium deficiency has been suspected in individuals with kwashiorkor, studies were undertaken by R. D. Montgomery (*Lancet* **II,** *74 (1960)*) to determine the magnesium levels in muscle and blood of infants with this disorder. He studied 27 infants, age nine to 24 months, with kwashiorkor or closely related clinical syndromes. Muscle biopsies were performed in 12 infants within ten days of admission and repeated five to nine weeks later while the individual was being repleted. He found that the average magnesium content of the muscle was 10.1 ± 0.56 mEq per kilogram wet weight on the first biopsy. Five to nine weeks after therapy a second biopsy indicated that the average magnesium level was 13.9 ± 0.87. The potassium content of the muscle at the first biopsy was 56 ± 3.56 mEq per kilogram of wet weight and the level rose to a value of 70 ± 4.19.

In a series of values in muscle of the normal individual this author listed data from four different investigators who reported that the average magnesium content varied from 16.3 to 20 mEq per kilogram wet weight and the potassium content ranged between 89 and 94. The fact that the magnesium levels obtained at the second biopsy are below the normal range may indicate that the patients still have not corrected their magnesium deficiency.

On measuring urinary magnesium it was found that for the first 40 days of study the average magnesium content ranged around 0.2 mEq per day on an intake of 1–3 mEq. Later the magnesium intake was increased to about 4–9 mEq per day and the urinary magnesium output increased to an average of 0.8 to 0.9 mEq per day. This investigator believes that the low urinary output during the first period is the result of both an increased rate of magnesium deposition into tissue and bone and a decreased amount absorbed from the gastrointestinal tract, possibly as the result of the diarrhea during the early period of therapy. No indication of the percentage of magnesium absorbed is given. On the other hand the average amount of potassium in the urine remained relatively constant after the fifth day with a tendency toward a little greater output during the later part of the study. The serum levels of magnesium were within the normal limits during the entire period.

From these studies, the author indicates that infants with kwashiorkor have a magnesium deficiency though no correlation was noted between the clinical state and the magnitude of the magnesium depletion. Also, no symptoms could be attributed directly to the magnesium deficiency.

FAT MOBILIZING AND KETOGENIC SUBSTANCES IN URINE

A potent fat metabolizing and ketogenic factor appears in the urine of individuals maintained on a fasting regimen or low calorie diet high in fat. The pituitary gland seems necessary for its production.

In 1947 R. Weil and D. Stetten (*J. Biol. Chem.* **168.** *129 (1947)*) found that an alkaline extract of urine of fasting rabbits injected into mice would increase the liver fat content. About ten years, later T. M. Chalmers, A. Kekwick, G. L. S. Pawan and I. Smith (*Lancet* **I,** *866 (1958)*) found that such a fat mobilizing activity could be isolated from the urine of man during periods of fasting. Later, they suggested that the potent factor responsible for this activity was peptide in nature.

Recently, Chalmers, Pawan and Kekwick (*Lancet* **II,** *6 (1960)*) reported a rather

simple scheme for isolating this material from urine of fasting human subjects. The procedure consists of adsorbing an alkaline extract of the benzoic acid precipitate of urine on oxycel and then eluting it with dilute sodium hydroxide. Yields up to 5 mg. of dried material were obtained from a 24-hour urine collection.

The extracts contained about 8 per cent nitrogen and following hydrolysis yielded seven different amino acids: histidine, phenylalarine, leucine, serine, cystine, aspartic acid and a trace of alanine. This material also gave a weak positive test for carbohydrate. Heating to 80°C in 0.1 normal sodium hydroxide did not reduce the biologic activity, though boiling for two minutes completely inactivated the product. The peptide was shown to have a molecular weight of less than 18,000. While some biological activity, remained after peptic digestion, all activity was lost following treatment of the extract with trypsin and chymotrypsin.

The potent biological activity of the material was evidenced by the fact that 10 μg. given subcutaneously to mice induced a large fall in blood sugar, a rise in blood ketone level, and increases in the fat content of the liver. One hour after administration of the fat mobilizing factor there was a slight increase in the blood sugar of the mice (an increase from approximately 82 to 90 mg. per 100 ml.). A precipitous drop then occurred with a minimum value of approximately 30 mg. per 100 ml. being reached by the fourth hour. This was followed by a slow rise in blood sugar which attained a level of 60 mg. per 100 ml. by the sixth hour after the injection.

The increase in the blood ketone level did not occur as rapidly as the changes in the blood sugar. Only slight changes were present from pretreatment level through the third hour. By the fourth hour a marked elevation in ketone bodies of the blood was found with the level being about three fold the initial value. The curve then tapers off with the sixth hour value being only a little greater than that noted on the fourth and fifth hours.

The liver fat in mice increased steadily with time following the injection. By the fifth hour after the administration of the fat mobilizing material the liver fat of the mouse almost doubled. On administering larger doses of the fat mobilizing factor (up to 200 μg.), increases in the blood lipids occurred. This included the total fat, phospholipid, cholesterol and the free fatty acid fractions. On a single large dose of the factor (0.4 to 40 mg.) the mice lost weight, which could be accounted for mainly by loss in fat and water. The preparation had a weak corticotrophic activity since it induced the excretion of steroids from the isolated adrenal gland.

The fat mobilizing and ketogenic activity was found in the urine of normal fasting individuals and those on low calorie regimens provided they received only little carbohydrate; it was not present in urine obtained from individuals on unrestricted diets or those on low calorie regimens containing high carbohydrate contents. Fat mobilizing and ketogenic activity has been noted in the urine of patients with diabetic ketosis, widespread malignant disease with wasting, and in two individuals with diffuse lipoatrophy. In a study of an obese patient on a reduction diet yielding 1000 calories per day (100 derived from protein) it was noted that the fat mobilizing factor did not appear in the urine until the fat supplied 50 per cent or more of the non-protein calories. When 90 per cent of the non-protein calories in this diet was supplied by fat, the fat mobilizing activity in the urine was maximum. The fat mobilizing activity was also found in the urine of fasted horses, goats, dogs and sheep.

On several occasions the effect of fasting or carbohydrate restriction was studied in six patients with well documented pituitary deficiencies. This group included individuals who had a hypophysectomy performed for the treatment of breast cancer or for other

therapeutic reasons. All the patients were receiving cortisone and thyroid as replacement therapy. When such individuals are placed on a 1000 calorie diet where 90 per cent of the non-protein calories was supplied by fat, no fat mobilizing or ketogenic activity was noted in the urine. Similar results were noted in hypophysectomized goats which were fasted up to five days.

Some of the chemical and physical properties of the fat mobilizing and ketogenic substance resemble corticotrophin or the anterior pituitary growth hormone. However the growth hormone is of larger molecular weight and is not adsorbed on oxycel. To compare the activity of the ketogenic material isolated from the urine with pig corticotrophic hormone the potency ratios of the two substances were determined by three different biological systems: (1) the release of steroids by the rat adrenal *in vitro*; (2) the release of fatty acids from the isolated rat epididymal fat pad; and (3) the increase in blood ketones in the intact unanesthetized mouse. The ratio of activity of the fat mobilizing factor to the pig corticotrophin for these three tests were 5/1000, 25/1000 and 10/100, respectively. This would indicate that the fat mobilizing or ketogenic factor is different from the pig corticotrophin. Also, after inactivating the corticotrophin with hydrogen peroxide it could be reactivated by cysteine. This did not occur with the urinary fat mobilizing factor.

These investigators believe that the fat mobilizing factor or the ketogenic substance is polypeptide in nature and appears to require the pituitary gland for its production. However, it does not appear to be either the pituitary growth hormone or corticotrophin. It is of interest that for activity in the urine the individual must be utilizing relatively large proportions of fat in his metabolic mixture. How this causes increased amount of the fat metabolizing and ketogenic activity in the urine is not known.

MULTIPLE ORIGINS OF MUSCULAR DYSTROPHY IN LAMBS

Muscular dystrophy, or "white muscle" disease, in lambs results from a number of dietary or environmental conditions other than a deficiency of alpha tocopherol and selenium.

The muscular dystrophy frequently seen in lambs has been recognized as a cause of "stiff lamb" disease, a condition noticed by shepherds since biblical times. During the last 30 years "stiff lamb" disease, or "white muscle" disease, has been attributed to a deficiency of vitamin E in the diet of the ewe during pregnancy. Still more recently it has been found that when sodium selenite is fed to ewes to supply 0.1 p.p.m. of selenium in the ration, muscle dystrophy may be prevented, even in cases where alpha tocopherol therapy has been ineffective (O. H. Muth, J. E. Oldfield, J. R. Schubert, and L. F. Remmert, *Am. J. Vet. Res.* **20,** *231 (1959)*). This indication that there are perhaps a number of dietary factors resulting in muscular dystrophy in sheep has renewed attention to natural feeding conditions and experimental diet (See *Nutrition Reviews* **17,** *235 (1959)*; **18,** *25, 90, 193, 227, 337 (1960)*).

A myopathy in sheep has been attributed to genetic background. F. D. Bosanquet, P. M. Daniel, and H. B. Parry (*Lancet* **II,** *737 (1956)*) have described a muscular disorder in older sheep (2.5 to 3.5 years old) suggesting that it is inherited and related to scrapie disease. On the other hand, where the muscular dystrophy occurs only in newborn lambs, some question may be raised as to the genetic origin of the condition.

J. T. Stamp (*J. Comp. Path. Therap.* **70,** *296 (1960)*) has described a number of conditions resulting in muscular dystrophy in sheep and has made an effort to relate these conditions to diet and to the develop-

ment of neonatal mortality in lambs. Stamp points out that there has been a great variation in the number of lambs involved from year to year. Thus, while in 1956 there was a very heavy loss of lambs, in 1957 the loss was very slight; in 1958 there was again a very considerable loss of lambs due to muscular dystrophy or to perinatal mortality, although the ewes were generally given a supplement of alpha tocopherol in the diet.

Lambs examined post-mortem showed muscles with soft, pale, poorly defined foci, although on occasion the lesions were more conspicuous due to the presence of hemorrhage, or chalky white streaks. The histological examination of the lesions showed that the muscle degeneration was similar to that described by others and indistinguishable from lesions caused by a vitamin E deficiency.

The muscular dystrophy and the perinatal mortality were associated with ewes on a diet pertaining to magnesium metabolism, ewes on a copper-deficient diet, ewes on "swayback", and lambs from ewes in the purebred herds which were being pastured. None of the ewes on the "swayback" hay produced dystrophic lambs. However, there was quite a large number of dystrophic lambs from the purebred Suffolks and from the Cheviot ewes on the copper-deficient diet and on the magnesium metabolism work.

Stamps made a careful examination of the livers from ewes and was unable to show a vitamin E deficiency although there was a wide variation in the vitamin E storage within the livers.

In post-mortem examination of ewes which had borne dystrophic lambs or had died because of lambs dying *in utero*, Stamp noted a very high incidence of muscular dystrophy, although he was unable to associate this directly to scrapie, or to the development of the dystrophic lambs. Thus, the dystrophy in the ewes did not directly correlate with the conditions observed in the lambs.

A very careful examination was made for any of the virus or bacterial diseases which might have been responsible for the conditions in the sheep and the lambs. None was found, and Stamp concluded that the neonatal mortality and the muscular dystrophy were of "environmental" origin rather than genetic. This conclusion was further strengthened by the observation that many of the ewes, apparently suffering from muscle dystrophy, recovered without treatment when grazing in the month following lambing.

This report by Stamp is especially interesting since it points out the multiple conditions which may be involved in the etiology of "white muscle" disease and the neonatal mortality which appears to be associated with this condition. This report is also interesting because of a tendency on the part of many investigators to attribute muscle dystrophy in lambs to either a deficiency of vitamin E or, more recently, to a requirement for selenium. It must be apparent that there are many possible causes of muscle dystrophy; some may be genetic, but many more may be due to either deficiency conditions, or the presence of ingredients of feed which interefere with normal metabolism of required vitamins or minerals in the diet. Much additional work will be needed to define the causes for such losses as Stamp described.

EXERCISE AND CALCIUM UTILIZATION

Enforced exercise in active adult dogs results in some decrease in apparent absorption of calcium, but it does not affect excretion or retention of this nutrient.

Enforced inactivity results in rapid and dramatic fall in skeletal calcium accompanied by increased urinary and fecal calcium excretion (*Nutrition Reviews* **6**, *146 (1948)*). Immobilization of a limb of a dog has been shown to cause atrophy of the bones of that leg proportional to the degree of disuse (N. Allison and B. Brooks, *Surg.*

Gynec. Obstet. **33,** *250 (1921)*). Such bone is thinner and lighter and has decreased breaking strength as a result of loss of substance. Exercise, even of minimal, passive nature, will ameliorate the tendency to lose calcium from bone of totally or partially immobilized subjects (*Nutrition Reviews* **7,** *295* (1949)). Thus, while inactivity results in loss of calcium from the body, exercise corrects this negative balance.

The effect of additional exercise on calcium utilization in subjects who are not on enforced inactivity is not as clear. In young growing dogs (J. A. Pinkos, cited in *J. Nutrition* **71,** *125 (1960)*) and in old dogs (C. H. Liu and C. M. McCay, *J. Gerontol.* **8,** *264 (1953)*) additional exercise has been shown to cause retention of calcium. The effect of additional enforced exercise on calcium utilization in young adult dogs has been further studied with the use of balance studies (F. Konishi and McCay, *J. Nutrition* **71,** *125 (1960)*). Also the utilizations of phosphorus, nitrogen, dry matter, ether extract, and carbohydrate were investigated.

In this investigation six adult, male, Beagle dogs averaging 9.0 kg. in weight and 22 months in age were housed in individual metabolism cages. They were fed a semisynthetic diet containing 0.17 per cent calcium and high levels of either carbohydrate, protein, or fat in order to test the additional effects of the latter nutrients on calcium utilization.

Exercise consisted of 30 minutes on a motor-driven treadmill at a gradient of 15.3 degrees and a speed of 6.0 km./hour. Three regimens of activity were used: a control period without exercise; exercise begun immediately after eating; and exercise begun one hour after eating.

Each dog was exposed to these three activity regimens while on a diet high in carbohydrate, protein, or fat. Animals were assigned randomly to a particular diet and to control and activity schedules. The diets were fed to the dogs for a period of 25 days before the experimental period so that they might become accustomed to them before beginning the balance study. Distilled water was allowed ad libitum. Determinations of calcium, phosphorus, nitrogen, dry matter, ether extract, and carbohydrate were made on diet and on urine and feces. Apparent absorptions were computed on the basis of intake minus fecal excretion and retentions were calculated as a percentage of the apparent absorption.

Exercise did not result in obvious ill effects to the dogs, and body weights remained relatively constant. A significant decrease in the apparent absorption of calcium occurred on the exercise regimens, but the apparent absorptions of phosphorus, nitrogen, dry matter, ether extract and carbohydrate were not affected. Urinary excretions and retentions of calcium, phosphorus, and nitrogen were not affected by exercise. The absorption of calcium was not affected by differences in diet and there were no interactions between diet and exercise. Urinary calcium excretions were highest in animals consuming the high protein diet and lowest in those fed the high fat diet.

As the authors indicate, the reasons for disagreement with other reports may be due to many causes and reflect the multiplicity of factors which are known to affect the absorption and utilization of calcium. Diverse dietary factors have long been known to influence calcium absorption. High levels of dietary protein have been found by some investigators to enhance absorption of calcium (R. A. McCance, E. M. Widdowson, and H. Lehmann, *Biochem. J.* **36,** *686 (1942)*).

Further study of this effect has indicated that the amino acids lysine and arginine are particularly active in promoting calcium absorption (R. H. Wasserman, C. L. Comar, and M. M. Nold, *J. Nutrition* **59,** *371 (1956)*; Wasserman, Comar, J. C. Schooley and F. W. Lengemann, *Ibid.* **62,** *367 (1957)*). Lactose and certain lactose-containing carbohydrates also increase intestinal absorption of calcium (*Nutrition Reviews* **18,** *154 (1960)*). The amount of fat in the ordinary diet may vary considerably and has negligi-

ble effect on calcium absorption (K. J. Jenkins and P. H. Phillips, *J. Nutrition* **70,** *241 (1960)*). It is probable that only when poorly absorbed does dietary fat adversely affect calcium absorption (Nicolaysen, N. Eeg-Larsen, and O. J. Malm, *Physiol. Rev.* **33,** *424 (1953)*). Thus, all three caloric nutrients may affect the absorption of calcium, and differences in dietary level in any or all of these nutrients may be responsible for some variation between experiments.

Dietary levels of other factors may also affect the efficiency of calcium absorption. Vitamin D is necessary for efficient absorption of calcium and its presence increases the rate of removal of calcium from the intestine (H. E. Harrison and H. C. Harrison, *J. Biol. Chem.* **185,** *857 (1950)*). The level of dietary calcium itself affects the efficiency of absorption and is more efficient at lower levels of intake (H. E. Harrison, *Fed. Proc.* **18,** *1085 (1959)*). Since calcium is most likely absorbed in the ionic form, intestinal absorption is decreased by those substances which reduce the concentration of ionic calcium in the intestinal lumen. A variety of anions which tend to form insoluble substances by precipitating or complexing calcium (such as oxalates, phytates and phosphates) act in this manner (Nicolaysen, *et al., loc. cit.*). An alkaline pH of the intestinal contents may potentiate these factors and the authors suggest that by causing inhibition of gastric secretion, exercise may increase the pH of the gut and thus inhibit calcium absorption.

Probably the most important difference between the experiments testing the effect of supplemental exercise on calcium absorption and utilization in young growing dogs, adult dogs and old dogs is the age of the animals and the consequent state of the skeleton in each case. In the young dog, active bone growth is taking place, whereas in the adult dog the bone is comparatively static and excess calcium is not needed for skeletal use in ordinary circumstances. In old animals, there often is considerable skeletal demineralization associated with the hormonal imbalances and/or a certain degree of inactivity of old age.

Since about 90 per cent of the body calcium is in the skeleton, the avidity with which this structure accepts calcium may affect the absorption and apparent utilization. Young growing animals absorb dietary calcium much more completely than older animals (S. L. Hansard and H. M. Crowder, *J. Nutrition* **62,** *325 (1957)*). Intestinal uptake is also greater in old rats whose skeletons have been depleted of calcium than in animals of the same age with normally calcified skeletons (Nicolaysen, *Acta physiol. scand.* **5,** *200 (1943)*). Thus, the extent of deposition of calcium into the skeleton and the state of mineralization of bone in some way influences the absorption of calcium by the intestine (H. E. Harrison, *loc. cit.*). Exercise superimposed on the skeletal condition in either the active young dog or the active old dog may be much more effective in stimulating the skeletal incorporation of calcium (and thus its utilization and absorption) than it is in the adult animal. In this way the young dog and the old dog more closely resemble the totally immobilized animal than does the adult dog.

This interesting report emphasizes the multiplicity of factors that can affect calcium absorption and utilization. Along with previous reports, it indicates that the degree of exercise may have some important effects on nutrient absorption and utilization.

STARVATION AND LACTIC DEHYDROGENASE

Parallel decreases in hepatic lactic dehydrogenase activity and nitrogen content occur during six days of starvation in the rat.

During starvation the body functions at the expense of its tissues. Storage forms of carbohydrate and fat are initially utilized, but with prolonged inanition protein is also

degraded and utilized for energy. In addition to preferential use of different caloric nutrients, there is also a preferential use of those from different tissues and organs. For instance, adipose tissue is rapidly lost due to utilization of fat, and glycogen and protein nitrogen are lost from liver more rapidly than from skeletal muscle, heart or brain (T. Addis, L. J. Poo, and W. Lew, *J. Biol. Chem.* **115,** *111 (1936)*).

Enzymes are proteins and previous studies have demonstrated that the activity of such hepatic enzymes as catalase, alkaline phosphatase, cathepsin, xanthine dehydrogenase, D-amino acid oxidase, arginase and succinoxidase decrease at the same rate as the total hepatic nitrogen (*Nutrition Reviews* **9,** *103 (1951)*). It was concluded that the hepatic enzyme proteins entered into the general metabolism and there was no indication that some were preferentially spared.

Maintenance of blood glucose is a problem of immediate importance in the starving animal. Subsequent to the utilization of available carbohydrate stores there develops decreased glucose tolerance and ketosis associated with gluconeogenesis from fat and protein.

Enzymes associated with the maintenance of blood glucose might be expected to be more important to the organism under the stress of starvation than some other enzymes. Indeed, it has been recently demonstrated that enzymes which take part in the production and utilization of glucose-6-phosphate react differently during starvation (G. Weber and A. Cantero, *Am. J. Physiol.* **190,** *229 (1957)*; *Exp. Cell Res.*, **14,** *596 (1958)*). Some enzymes decrease at the same rate as hepatic nitrogen, whereas others are preferentially maintained or further depressed (Weber, *Rev. Canad. Biol.* **18,** *245 (1959)*). Thus, at the enzymatic level there is evidence of preferential maintenance of essential elements.

The present study extends the investigations of enzymatic changes in starvation to lactic dehydrogenase which is involved in an important pathway of pyruvate metabolism (Weber, *J. Nutrition* **71,** *156 (1960)*).

In this investigation, male Wistar rats weighing 180 to 220 g. were caged separately and fed laboratory chow and water ad libitum. Experimental animals were treated similarly except that they were fasted for periods of one, three and six days, being allowed only water. Each group of control and experimental animals contained at least four rats.

Animals were sacrificed by decapitation, and bled, and 10 per cent homogenates of liver were prepared in isotonic KCl solution. The number of nuclei per ml. of homogenate was counted. Supernatant fluid was separated by centrifuging liver homogenate for 30 minutes at 100,000 × g. and 0°C. Lactic dehydrogenase activity was determined on the supernatant fluid. The course of the reaction was followed by determining the decrease in optical density due to DPNH at 340 mμ at 30-second intervals for a period of 30 minutes. Reaction cuvettes contained a mixture of the following reagents in final molarity: sodium pyruvate (2.5×10^{-4} M); DPNH (2.5×10^{-5} M); phosphate buffer (0.2 M), pH 7.4; sufficient enzyme to give linear kinetics over a period of ten minutes at 37°C.; and water to give a final reaction volume of 3.0 ml. The blank contained all reagents except DPNH. Activity was expressed as micromoles of pyruvate converted into lactate; a unit of activity was expressed as the amount of enzyme which produces one micromole of lactate per gram wet weight of liver at 37°C. Nitrogen was determined by the microKjeldahl procedure. Enzymatic activities were expressed on the basis of per average cell and per 100 g. of body weight.

During six days of fasting, the rats lost 32 per cent of their initial body weight. Liver weight decreased to 39 per cent of the initial control value and the ratio of liver weight to body weight fell to 64 per cent of that in control rats. The number of nuclei per gram wet weight of liver gradually increased to 191 per cent of the control value at the end of the six days of fasting.

The nitrogen content of the liver expressed as the content of the average liver cell decreased to 76 per cent of normal at one day of fasting, to 68 per cent at three days, and to 54 per cent at six days. Lactic dehydrogenase activity decreased at a parallel rate giving comparative values of 74 per cent, 61 per cent, and 54 per cent of the control level at one, three, and six days of fasting, respectively. When expressed on the basis of body weight, nitrogen decreased to 59 per cent of the control value at one day, 51 per cent at three days, and 34 per cent at six days. Comparative values for lactic dehydrogenase activity were 56 per cent, 45 per cent, and 34 per cent of the control levels.

Thus, lactic dehydrogenase activity is not preferentially maintained during starvation. It has previously been reported that glucose-6-phosphatase is maintained above the decreasing nitrogen level while phosphoglucomutase and phosphohexoseisomerase fall parallel with it (Weber and Cantero, *loc. cit.*). Another interesting observation is that during six days of starvation, which for the rat is severe, no enzyme decreased below 30 to 50 per cent of the control value.

These facts suggest that an excess of some enzymes, such as phosphoglucomutase, phospho-hexoseisomerase and lactic dehydrogenase is present and that a decrease in their activity by as much as 50 to 70 per cent can occur without affecting physiological functions (Weber and Cantero, *loc. cit.*) while others that are rate limiting because of their amount or activity must be comparatively maintained at normal levels.

These interesting and stimulating observations must be evaluated in the light of knowledge of the cytology and histochemistry of the liver. The liver is not a homogeneous organ from either cytologic or chemical aspects. Approximately 30 to 40 per cent of all the cells in the normal liver are other than hepatic parenchymal cells (M. Abercrombie, *Anatomical Record* **94,** *239 (1946)*). These nonparenchymal cells are strikingly different from parenchymal cells in cytologic and enzymic constitution, and they are not uniformly distributed throughout the hepatic lobule. They may not react to starvation or to other influences in a manner either qualitatively or quantitatively analogous to parenchymal cells. The concept of the average hepatic cell based on data from homogenates of whole liver is artificial.

Furthermore, there is a considerable body of evidence that parenchymal cells located in different parts of the hepatic lobule vary considerably from the standpoints of both structure and function. For instance, mitochondria in the periportal areas are thin and filamentous, while those in the centrolobular areas tend to be shorter and more plump (D. Fawcett, *J. Nat. Cancer Inst.* **15,** *1475 (1954)*). Glycogen is preferentially maintained in the portal areas (H. Deane, *Anatomical Record* **88,** *39 (1944)*; and different etiologic types of fat infiltration tend to affect characteristic zones of the lobule.

These cytologic differences are probably the result of variations in the levels of different enzymes within the hepatic lobule. Indeed, methods of histochemistry using sections of liver observed microscopically (A. B. Novikoff, *J. Histochem. & Cytochem.* **7,** *240 (1959)*; or homogenates of a few cells dissected microscopically from different parts of the hepatic lobule (R. E. Shank *et al.*, *Ibid.* **7,** *237 (1959)*) have demonstrated marked differences in the localization of particular enzymes in the cells from one part of the lobule to the other.

For instance, lactic dehydrogenase, the enzyme under discussion, is concentrated mainly in the periportal and midlobular areas, whereas the level of nitrogen is equal in all zones of the hepatic lobule (Shank *et al.*, *loc. cit.*). Some noxious agents may affect an enzyme in one part of the lobule more than in another. It is not known whether starvation affects lactic dehydrogenase activity in all parts of the lobule equally. Homogenate techniques will not allow this distinction to be made and may mask some striking and potentially important lobular alterations.

This criticism is not directed at the author

of this interesting and provocative paper and in no way is meant to imply that data from homogenate techniques are useless. The recent advances in the field of biochemistry belie this. However, it is a plea for more general and extensive use of cytochemical and histochemical methods in conjunction with homogenate techniques. With the general recognition that the liver is not a homogeneous organ there may come detection of subtle and possibly important spatial alterations in enzymes and other substances as a response to various influences. These facts may help us to gain further knowledge of the function of this important and complex organ.

EFFECTS OF DIET AND AGE ON INTESTINAL FLORA IN THE PIG

The concentration of intestinal bacteria in the feces of the pig is partially dependent on the type of diet, the age of the animal, and the environment and manner of feeding.

Intestinal microorganisms are of great importance because of their ability to synthesize vitamins and other food factors essential to mammals (O. Mickelsen, *Vitamins and Hormones* **14,** *1 (1956)*). The intestinal flora may vary considerably both quantitatively and qualitatively because of dietary and other variables. A recent article has explored the effect of variations in the type of carbohydrate in a semisynthetic diet, in the environment, in the manner of feeding, and in the age of the animal on the type and quantity of bacteria in different parts of the gastrointestinal tract of the pig (R. D. Wilbur *et al., J. Nutrition* **71,** *168 (1960)*).

In this study pigs of both sexes from different litters were farrowed and reared to two weeks of age in stalls with concrete floors. During this time they had access to the sow's feces, water, and food supplies. The pigs were vaccinated against hog cholera and erysipelas. Male pigs were castrated at five days of age. At two weeks they were removed from the sows and placed in air-conditioned quarters isolated from other pigs. Feed and water were offered ad libitum and pens were cleaned daily. The pigs were randomly assigned to two groups, each containing 24 animals. One group was fed a semisynthetic diet containing lactose and the other group a similar diet containing starch as the source of carbohydrate. No antibiotics were administered. Half of the pigs in each group were individually penned and the remainder were penned in groups of six. The experimental period consisted of 28 days (14–42 days of age). Body weight, feed consumption, and fresh rectal fecal samples were studied at weekly intervals. Half of the pigs were sacrificed at 42 days of age and feces were collected from the duodenum, ileum, cecum, and rectum for bacterial studies. Samples of feces were handled individually and standard plate count techniques were used. Each sample was diluted in nine parts of sterile buffered water and the mixture was blended. Serial dilutions of this mixture were made in buffered water. Each plate was inoculated with 1.0 ml. of the appropriate dilution and 18 ml. of culture medium. Organisms studied with the use of special, selective media were: total aerobes, total anaerobes, lactobacilli, coliforms, streptococci, staphylococci, and molds and yeasts.

Pigs gained an average of 5.77 kg. during the four weeks of the experiment. Both the weight gain and the feed required per kilogram of gain were greater in those animals fed the diet containing lactose. Individual-fed pigs gained at a greater rate than did group-fed animals (6.26 kg. as compared to 5.31 kg.).

All rectal bacteria studied were significantly decreased in number by dietary lactose as compared to starch except for total anaerobes and lactobacilli. Significant differences in total aerobes, total anaerobes, staphylococci and molds and yeast occurred between litters. In general, with time there were increasing population densities of all

bacteria studied. However, in pigs fed the diet containing lactose there was a primary decrease in coliforms, staphylococci, and molds and yeasts. Individual-fed pigs had lower counts for all organisms studied than group-fed pigs, but this was statistically significant only for total aerobes, total anaerobes, and lactobacilli.

The authors conclude that diet as well as age and diet interactions had predominant effects on the total aerobes, coliforms, streptococci, staphylococci, and molds and yeasts. Management (*i.e.* group or individual feeding) was apparently the controlling influence on total anaerobes and lactobacilli. It was considered that dietary effects were great enough to mask the effects of management for the majority of organisms. In general, counts of organisms varied somewhat between different segments of the intestines, but there was a marked increase in concentration of all bacteria from proximal to distal intestine.

In most regions of the intestine the diet containing lactose caused a lower count of all organisms compared to animals fed the diet containing starch. This was especially marked for streptococci, staphylococci, and molds and yeasts. There were some variations between litters, and the numbers of lactobacilli and streptococci were higher in group-fed animals than in those individually fed.

In discussing these results the authors make several observations. Pigs can tolerate a diet containing a high per cent of lactose. The higher weight gains in the individual-fed group may be due to less competition for food. They also advance the view that it may be due partially to fewer intestinal bacteria in these animals to compete with the organism for food. It is possible that the higher bacteria count in group-fed pigs is due to the greater opportunity to eat feces and to the constant reinoculation of fecal bacteria thus imposed. The change in population density of bacteria at weaning is considered to be due mainly to change in substrate in the food. This is reasoned because the sow's milk contains a high content of lactose and thus the change was not as marked in those receiving the diet containing lactose. The tendency for an increased concentration of bacteria over the experimental time period may be the result of changes in intestinal enzymes and other secretions. The tendency for increasing count from proximal to distal gut is not entirely explicable on the basis of fecal concentration and probably depends in part on changes in secretions or differential rate of bacterial turnover.

As the authors point out, one of the major problems in considering intestinal or fecal microorganisms is the variation induced by the experimental procedures used. This study indicates the way to reduce these variables by the use of purified diets and standardized techniques.

DRUGS WHICH INDUCE ASCORBIC ACID SYNTHESIS IN RATS

A number of drugs of diverse origins are capable of increasing synthesis and excretion of ascorbic acid in rats.

It has been more than 20 years since the discovery that a number of drugs caused an increased rate of synthesis and excretion of ascorbic acid in rats (H. E. Longenecker, R. R. Musulin, R. H. Tully, III, and C. G. King, *J. Biol. Chem.* **129,** *445 (1939)*; *Nutrition Reviews* **12,** *155 (1954)*). This finding led to evidence indicating that none of the substances tested (barbiturates, cyclic ketone, and analgesics) served as precursors of ascorbic acid. Much of the information relative to synthesis of ascorbic acid has resulted from the work of King and his co-workers. They demonstrated that the most probable route of synthesis of ascorbic acid was from D-glucose to D-glucuronic acid to L-gulono-

lactone to L-ascorbic acid (*Nutrition Reviews* **12,** *215 (1954)*). Studies of the ability of fresh liver taken from rats or from guinea pigs to convert L-gulonolactone, labeled with carbon 14, to L-ascorbic acid, demonstrated that rat liver had this property but guinea pig liver did not (*Nutrition Reviews* **15,** *160* (1957)).

Recently, J. J. Burns *et al.* (*J. Pharmacol. Exp. Therap.* **129,** *132 (1960)*) reported their observations on the synthesis of D-glucuronic, L-gulonic and ascorbic acids by rats given a number of different drugs. Employing radioactively tagged precursors of ascorbic acid, they studied in male albino rats the rate of urinary excretion of D-glucuronic acid and L-ascorbic acid during the administration of various drugs. These included phenylbutazone, several polycyclic hydrocarbons, and the antihistaminic drugs, diphenhydramine and chlorcyclazine. The possibility of altered rate of excretion due to a renal effect of such drugs had been excluded previously by studies of nephrectomized rats.

Administration of meprobamate, diphenhydramine, chlorcyclazine and several polycyclic hydrocarbons resulted in substantial increases in the rate of excretion of L-ascorbic acid in the urine of these rats. The previously reported effects of phenolbutazone, aminopyrine and antipyrine in augmenting the excretion of L-ascorbic acid were confirmed by this study. Of particular interest was the fact that the carcinogenic hydrocarbons, 3-methylcholanthrene, 1,2,5,6-dibenzanthracene, and 3,4-benzpyrene had a similar effect upon ascorbic acid.

Many other drugs have been reported previously to have an influence upon the rate of ascorbic acid synthesis and urinary excretion (Longenecker, H. H. Fricke, and King, *J. Biol. Chem.* **135,** *497 (1940)*). These included such diverse drugs as urethane, acetanilid, phenacetin, paraamino phenol, sulfanilamide, codeine, quinine, and salicylates. Studies of the rates of formation of D-glucuronic acid, of L-gulonic acid and of ascorbic acid showed that some drugs produced a disproportionate increase in the concentrations of one or another of these metabolites. For example, barbital increased the excretion of both D-glucuronic acid and ascorbic acid yet borneol and aniline increased production of the glucuronides but not of gulonic acids or of ascorbic acids.

Recently, a new route for ascorbic synthesis has been reported (O. Touster, *Am. J. Med.* **26,** *724 (1959)*; Burns, *Ibid.* **26,** *740 (1959)*; J. L. Strominger, *Physiol. Rev.* **40,** *55 (1960)*). According to this scheme, D-glucose is oxidized to form D-glucuronic acid which is reduced to L-gulonic acid. This compound can then be converted to L-ascorbic acid or to L-xylulose. Apparently certain drugs can alter the rate of conversion of glucose toward the glucuronic acid pathway and therefore alter the rate of synthesis of ascorbic acid or of its other metabolites. Antipyrine and aminopyrine, which stimulate ascorbic acid synthesis, also increase the rate of urinary excretion of L-xylulose.

This study adds additional information regarding the metabolism of ascorbic acid by demonstrating that drugs of diverse nature can alter the rate of formation of ascorbic acid and other metabolites. This may be of value in interpreting enzymatic reactions which involve ascorbic acid synthesis.

SUBSTANCES INFLUENCING THE ARGININE REQUIREMENT

The arginine requirement of chicks is much higher when casein rather than soybean meal is the source of protein. Small amounts of soybean meal reduce the requirement for arginine when a casein ration is fed, even in the presence of an excess of glycine, creatine and creatinine.

It has been recognized for a number of years that when chicks are fed a purified diet containing casein as the source of protein, the diet must be supplemented with arginine

to secure maximum rates of body weight gain (*Nutrition Reviews* **15,** *43 (1957)*). Later, it was shown that a number of compounds related to creatine would reduce the chick's requirement for arginine. The chick also requires a dietary source of glycine and one of the sulfur amino acids (methionine is used most frequently) for maximal body weight gains.

Inadequate levels of arginine in the ration produces not only poor growth but also poor feathering and a "high-stepping, stilted gait" (*Nutrition Reviews* **17,** *44 (1959)*). The requirement for arginine and glycine can be met by using a combination of casein and gelatin.

Calculations indicated a marked difference in the arginine requirement of chicks fed purified rations and commercial stock rations (composed primarily of corn and soybean meal). This was true even though both rations produced the same rates of body weight gains.

Some of this work was done by B. A. Krautmann and co-workers (*Poultry Sci.* **36,** *935 (1957)*), who calculated that on a purified diet composed of casein and gelatin chicks required 1.67 per cent arginine for maximum rates of body weight gain. When these investigators used a purified diet containing only casein at a 21 per cent level, they had to add enough arginine to bring the level up to 1.65 per cent for maximum rates of growth. The addition of about 9 per cent gelatin to the 21 per cent casein diet permitted a reduction in the arginine level to 1.39 per cent. The reduction in the arginine requirement was attributed to the extra glycine provided by the gelatin. When 58 per cent ground corn was used in the ration in place of dextrose, maximum growth was secured with 1.25 per cent arginine in the diet. The best growth rates were secured on a practical-type ration containing 1.15 per cent arginine. The addition of extra arginine to the latter produced no further increase in weight gains.

These workers concluded that there is an unidentified factor in plants which enhances the utilization of the arginine in casein or otherwise makes the amino acid more available to the chick. They found that when 4.7 per cent soybean meal was added to the purified diet containing 0.80 per cent arginine, the four-week weight gain of the 15 male chicks was 150 g. (Krautmann *et al.*, *Poultry Sci.* **37,** *530 (1958)*). This was three times the weight gain of the controls fed the 22 per cent casein diet supplemented with 1 per cent glycine and 0.50 per cent methionine. The investigators were so impressed with the effect produced by the low levels of soybean meal that they suggested the presence of a vitamin-like substance in plants. The soybean meal contributed 2 per cent protein and, according to Krautmann and co-workers, "small changes in amino acids do not produce gains of this magnitude." The sum of the creatine and creatinine in the soybean meal was so low (<0.05 per cent) that it could not explain the arginine-sparing action.

Replacing part of the dextrose in the purified diet with 40 per cent whole corn produced weight gains that were as good or slightly better than those secured with 4.7 per cent soybean meal supplemented ration. When 4 per cent corn germ was used in place of the whole corn, the gain in body weight was 111 g. (versus 173 g. for the ration containing 40 per cent whole corn). It should be pointed out that these rates of growth are much less than 363 g. observed when chicks were fed practical-type rations containing 1.1 per cent arginine (Krautmann *et al.*, *Poultry Sci.* **36,** *935 (1957)*).

The addition of 0.5 per cent sulfasuxidine to the above rations had no effect on the growth rates. For this reason, Krautmann *et al.* concluded that intestinal microbial action was probably not associated with the effect.

Work along a related line has been carried out by J. E. Savage and B. L. O'Dell (*J. Nutrition* **70,** *129 (1960)*). They fed their day-old chicks a purified-type diet contain-

ing 35 per cent casein, 10 per cent soybean oil, and glucose as the source of carbohydrate. One diet contained 1.5 per cent supplemental glycine. When graded levels of arginine were added to the two diets, greater growth was secured at all levels on the glycine diet. At four weeks the average weight of the chicks on the glycine diet (containing 2.4 per cent arginine) was 38 g. greater than that of the group receiving the same diet without the glycine; this difference was significant at the 1 per cent level.

The addition of creatine, creatinine, or guanidoacetic acid to the purified diet containing 1.5 per cent glycine permitted a reduction in the arginine content of the diet for maximum growth. The guanidoacetic acid was less effective than either creatine or creatinine in sparing arginine; this was true whether extra glycine was or was not added to the diet. Creatine stimulated the growth of the chicks only when the level of arginine was too low for maximum growth. Under these circumstances, 0.3 per cent creatine would spare an equal weight of arginine.

It was suggested by Savage and O'Dell that the preformed creatine reduced the arginine requirement by eliminating the chick's need for arginine in synthesizing creatine. It is unfortunate that these investigators did not compare the activity of the above compounds with soybean meal since low levels of this meal will markedly reduce the requirement for arginine.

The above work is important in showing that the chick's requirement for arginine is influenced by a number of factors. The marked difference in the arginine requirement when purified and practical-type rations are used may be worthy of more intensive study since Krautmann and coworkers (*Poultry Sci.* **36,** *935* (*1957*)) stated that for chicks "much lower levels of tryptophan are sufficient in practical diets than have been indicated when purified diets were employed."

One might think of plant substances, such as canavanine, as possible sparers of arginine. Canavanine is an amino acid which has a structure similar to that of arginine. It occurs in many plants but was first isolated from jack bean. It is about twice as active as arginine (J. F. Van Pilsum, D. A. Berman and E. A. Wolin, *Proc. Soc. Exp. Biol. Med.* **95,** *96* (*1957*)) in transamidative reactions involving glycine when rabbit liver served as the source of the enzymes. It is also active when the enzyme was prepared from hog kidney tissue (S. Nakatsu, *J. Biochem.* **43,** *675* (*1956*)). On this basis, it has been suggested that it is not a normal metabolite of animal tissue but, once it has been ingested, it can serve in place of arginine in a number of enzymatic reactions. In contrast to this is its growth inhibitory power for many strains of bacteria where it has been classified as an antagonist (B. E. Volcani and E. E. Snell, *J. Biol. Chem.* **174,** *893* (*1948*)).

A more plausible explanation may involve the chick's inability to utilize the arginine in casein as efficiently as it does when other proteins are used in the ration. Confirmation of this suggestion comes from the observation of G. J. Klain, H. M. Scott and B. C. Johnson (*Poultry Sci.* **39,** *39* (*1960*)) that when chicks are fed on a diet containing only amino acids in place of protein, the arginine requirement is 1.08 per cent. Admittedly, the chicks in the latter work were maintained on the amino acid diet for only seven days so that it is difficult to evaluate the different requirements reported for arginine. The level of 1.08 per cent is, however, very similar to the 1.1 per cent for the practical-type corn-soybean meal ration used by Krautmann *et al.* (*Ibid.* **36,** *935* (*1957*)).

With this possibility in mind, it might be advisable to check the digestibility both *in vivo* and *in vitro* of casein versus the proteins in soybean meal. This work obviously should be done with the chick, but, for comparative purposes, it might also be advisable to do the same thing with the rat.

The work of E. L. Hove and J. F. Herndon

(*J. Nutrition* **63,** *193 (1957)*) suggests that casein might not be as digestible in an herbiverous animal as the plant proteins. These investigators found that when rabbits were fed a purified diet containing 18 per cent they gained an average of 4 g. per day. When an enzymatic digest of casein was used in place of the casein, the daily weight gain increased to 15 g. The rabbit is somewhat similar to the chick in its growth response to casein and soybean meal. Hove and Herndon observed that weanling rabbits fed a purified 20 per cent casein diet containing added potassium grew at the rate of 10 g. per day. The addition of 15 per cent soybean meal (51 per cent protein) to this ration in place of an equal weight of sucrose increased the weight gain to 26 g. This was still less than the 34 g. per day secured on the commercial rabbit ration containing 18 per cent protein.

If additional work is done in this area, it might be advisable to compare the growth response secured when chicks are fed a casein diet supplemented with glycine, arginine and methionine with that secured both with a practical soybean meal-corn ration and a purified-type diet containing one of the purified soy proteins. Finally, the influence of the mineral mixtures in the different diets should be carefully considered since G. M. Briggs (*Poultry Sci.* **35,** *740 (1956)*) has suggested that the growth response secured with many natural products might be due to the addition of minerals in which the basal diet was deficient.

VITAMIN E-FREE DIETS IN CHICKS

Chickens grow normally and develop no symptoms of vitamin E deficiency on a vitamin E-free diet if sufficient vitamin A, selenium, and an otherwise adequate diet are provided.

Whether vitamin E has some specific metabolic functions or owes its entire action to its antioxidant properties has been a subject for investigation and discussion for many years. For example, although several antioxidants have been found to replace tocopherol in many of its functions, F. Christensen and H. Dam (*Nutrition Reviews* **15,** *116 (1957)*) found that the efficacy of methylene blue as a substitute for tocopherol in rats lay in its protection of very small amounts of tocopherol present in the diet.

Moreover, B. G. Creech and co-workers (*Nutrition Reviews* **17,** *154 (1959)*) found that the onset of exudative diathesis in chicks (a typical symptom of vitamin E deficiency produced in this case by a diet containing torula yeast as the protein source) was not accelerated by dietary lard, leading to the conclusion that this symptom is not associated with the antioxidant properties of vitamin E. Other examples of an apparent specific function of vitamin E can readily be found.

Still, in more recent years, the results of many studies seem to lead to the opposite conclusion. For example, the work of L. J. Machlin, R. S. Gordon and K. H. Meisky (*Nutrition Reviews* **17,** *340 (1959)*; **18,** *95 (1960)*) indicates that all the symptoms of vitamin E deficiency can be produced by omitting vitamin E or other antioxidants (and in some cases, selenium or sulfur amino acids) from the diet and, usually, including polyunsaturated acids; the same symptoms can be prevented with adequate dietary antioxidant alone. In particular, finding that selenium can prevent many of the symptoms of vitamin E deficiency has led to experiments which may answer these questions.

One such study is that of J. G. Bieri, G. M. Briggs, C. J. Pollard and M. R. Spivey Fox (*J. Nutrition* **70,** *47 (1960)*). The experiments were carried out on chicks raised from one day on diets with no added antioxidants. Since difficulty was experienced in preventing vitamin A oxidation with such diets, this vitamin was given weekly as a precaution shown to be of some importance in this type of study.

In the first experiment, the protein source was 20 per cent purified casein plus 8 per cent gelatin with additional 0.3 per cent DL-methionine. Chicks raised for 24 weeks on this diet showed normal growth and development even though serum vitamin E was undetectable. When 10 per cent vitamin E-free lard was added to the diet, growth was superior to that of groups of chicks provided with supplementary tocopheryl acetate or tocopheryl acetate and 4 per cent corn oil. In no case did the usual symptoms of vitamin E deficiency in chicks (exudative diathesis and encephalomalacia) appear. The authors give two reasons for this: first, the casein contained enough selenium to prevent exudative diathesis; second, it was found that at least 15 per cent lard was necessary for development of encephalomalacia.

The second experiment was carried out over a year and differed in that the diet contained 30 per cent soybean protein as the protein source and 4 per cent vitamin E-free lard. L-Cystine was included as a source of sulfur amino acids. Sodium selenite (0.5 p.p.m.) was added to prevent exudative diathesis and 30 per cent torula yeast was included during the first six weeks, but was discontinued when it appeared to cause watery excreta. Growth and appearance of birds on this diet were apparently quite normal and comparable to those of a similar group with a vitamin E supplement (50 mg. alpha-tocopheryl acetate per kg.). Egg production was not good in either group but fertile eggs could be produced by artificial insemination. Hematocrit and hemoglobin values were similar in both groups and total serum proteins were comparable.

The authors found that although apparent liver vitamin E levels of the deficient group dropped to a low level within four weeks, no further reduction occurred throughout the duration of the experiment. Actually, the material analyzed in these latter periods proved to be other related substances, such as reduced coenzyme Q, and vitamin E was essentially absent from the tissues and eggs of the birds on the deficient diet.

In view of the results of Christensen and Dam (*loc. cit.*) the authors investigated the possibility that minute amounts of vitamin E might have been present in the diet. Since the amount was below the limits of their analytical procedure, it appears that the chickens were getting no vitamin E unless it was given as a supplement.

The conclusions reached from these experiments are that no dietary vitamin E is necessary for normal growth and development of chicks on these diets if sufficient vitamin A is provided, and that the primary function of vitamin E may lie in the protection of vitamin A. Evidently other antioxidants in the diet or produced in the body prevented the accumulation of harmful peroxidized products. These may actually be lipid-soluble substances such as vitamin A and coenzyme Q or water soluble compounds such as selenium-containing materials or the sulfur amino acids.

Finally, the authors conclude that these studies seem to rule out a vital role for tocopherol in electron transport or other phases of intermediary metabolism.

FAT ABSORPTION AND UTILIZATION IN TUMOR-BEARING RATS

Iodine^{131}-labeled lipids were used to study fat absorption, clearance and tolerance in tumor-bearing rats compared with their pair-fed controls in an attempt to determine the cause of hyperlipemia and cachexia.

The cause of anorexia, body weight loss, depletion of depot fat and cachexia in tumor-bearing rats has not been well understood. Force-feeding experiments attempting to prevent weight loss cause an exaggeration of hyperlipemia. The possibility that the total energy expenditure of tumor-bearing animals is increased, coupled with an

inability of tumor tissue to synthesize fatty acids fast enough, could account for the need to secure fat from the host. Hyperlipemia could be due either to mobilization of fat or defective clearance.

Three types of studies using I^{131}-labeled lipids have been reported by I. Posner (*Cancer Research* **20,** *551 (1960)*): fat tolerance studies, *in vivo* and *in vitro* clearance studies, fat absorption studies.

Male albino rats were fed a normal complete diet for a period of seven to ten days, then divided into comparable groups of three rats per group on the basis of diet consumption and weight gain. One animal, the normal control, was fed ad libitum; one of the other two rats was given Walker carcinoma and the other became the pair-fed control being fed only as much as the tumor-bearing rat ate the previous day.

Iodine^{131}-labeled triolein was given in quantities of 10 microcuries per rat. Care was taken to use identical rations with pair-fed animals because of the difficulty in achieving a constant concentration of labeled triolein in the diet.

The fat tolerance test was done by force-feeding triolein-I^{131} and collecting blood at 1, 2, 3, 4 and 6 hours after the feeding. Plasma was mixed with 15 parts unlabeled plasma to facilitate recovery. After precipitation of plasma chylomicrons and lipoproteins, free I^{131} was removed and radioactivity of the residue was counted. Results of fat tolerance and absorption were expressed as per cent total dose (TD). A plot of per cent TD vs. time in hours gave the "fat tolerance curve."

Cannulae were also inserted in the thoracic ducts of all animals two to four hours after force feeding 0.1 ml. of olive oil. Twenty four hours later triolein-I^{131} test diet was fed and the lymph as well as plasma was collected and processed. The lymph that had been collected from triolein-I^{131}-fed rats was injected into the femoral vein of fasted rats and blood was collected at intervals for analysis as before. Per cent clearance was determined by dividing the activity at any one time by the initial plasma activity and multiplying by 100. Per cent clearance was plotted against time in minutes.

In vitro clearance was studied by using plasma, the source of the lipase system, collected from the dorsal aorta three minutes after heparin injection and mixing it with absorptive lymph as a substrate.

The results of fat tolerance were plotted as graphs of average activity (per cent of total dose) against time in hours. Peak values of normal rats were compared with peak values of tumor-bearing animals.

Normal rats weighing about 150 g. had peak values averaging 24 per cent of the total dose while rats weighing about 260 g. had peak values averaging about 14 per cent TD. Several groups of large tumor-bearing rats (175–353 g.) were studied at different stages of tumor growth. Peak values of activity (per cent TD) for each group of animals bearing nine-day, 14-day and 21-day old tumors were plotted against time. The longer the tumors grew, the greater the activity of plasma lipids associated with extreme hyperlipemia.

Small rats weighing between 125 and 150 g. were injected with tumors and subjected to fat tolerance studies along with their pair-fed controls. The response in the small rats bearing tumors was similar to that mentioned for the large animals with tumors. For example, by the fourth week of tumor growth the tumor animals weighed an average of 255 g. and the pair-fed controls averaged 207 g. weight. The peak of the mean tolerance curve was 80 per cent TD per 100 ml. plasma which was more than three times the peak of the normal mean tolerance curve of the larger rats and eight times as high as the mean of the pair-fed control group.

The turbidity of the plasma of tumor-bearing rats was always greater than that of the normals or pair-fed control animals and was present even after fasting.

Study of thoracic duct lymph after a diet containing triolein-I^{131} revealed that tumor-

bearing rats absorbed much less fat than did the normal or pair-fed controls. Large rats absorbed more fat than did small rats in the same group. Tumor-bearing rats of the larger size absorbed about one half the amount of fat absorbed by pair-fed controls and about three and one half times the amount of fat absorbed by small rats with tumors.

Radioactively-labeled lymph was injected and tail vein blood was sampled at intervals in order to compare the rates of clearance of lipids in normal versus tumor-bearing rats. When citrated, diluted lymph was injected, the concentration of uncleared lipid in the plasma of tumor-bearing rats was three times higher than it was in the plasma of pair-fed control rats. When heparinized, undiluted lymph was used, the concentration of lipid was ten times higher than the controls.

In vitro clearance studies comparing lipoprotein lipase activity in pair-fed control rats with that of tumor-bearing rats showed that there was essentially no lipoprotein lipase activity in the latter group.

In discussing the hyperlipemia of cancer the author distinguished between post absorptive and absorptive hyperlipemia. Plasma lipids seem to be maintained in a steady state after absorption. During absorption, however, the hyperlipemia is alimentary and the tolerance curve represents a state of change. Tolerance depends on the relationship between the rate of absorption and the rate of clearance or utilization. In the tumor-bearing rats the peak of the mean tolerance curves was strikingly elevated. The extent of this effect appeared to be directly related to the tumor growth. In the last week of tumor growth nearly all the fat absorbed could be accounted for in the plasma at the time of the peak in the tolerance curve. In contrast, the tolerance peak of pair-fed control rats declined to levels below normal beyond the second week.

The amount of fat absorbed in the three groups of rats was studied. The absolute amounts of fat absorbed by large rats exceeded the amounts absorbed by small rats. However, when the amount was calculated as per cent of total dose per gram of body weight the difference was not remarkable. Tumor-bearing small rats absorbed less fat than tumor-bearing larger rats and were characterized by more pronounced anorexia, weight loss, hyperlipemia, rate of tumor growth and cachexia. Tumor-bearing rats absorbed less fat than their pair-fed controls in both age groups. The rate of absorption was also less.

In vivo clearance studies indicated a much slower clearance of fat by tumor-bearing animals after a single injection. A second injection resulted in a much higher "initial" concentration of lipides and a greatly delayed clearance compared with pair-fed control animals.

Heparin-clearing factor was not present in the plasma of tumor-bearing rats according to the *in vitro* studies. Absorbed fat tended to accumulate in the plasma and this phenomenon continued as tumor growth progressed.

Several factors appear to be responsible for the cachexia associated with cancer: anorexia with its resultant decrease in food consumption, the decrease in fat absorption, and the inefficient utilization and clearance of fat from the blood.

PHYSICAL ACTIVITY AND EXPERIMENTAL DIETARY ATHEROSCLEROSIS

Rabbits fed a high cholesterol diet were divided into an exercised and a sedentary group. Comparison was made as to the amount of atherosclerosis, and aorta and serum levels of cholesterol, cholesterol esters, lipid phosphorus and cholesterol to lipid phosphorus ratio.

It is only recently that physical activity has been considered as a possible factor in the development of experimental and human atherosclerosis. Much interest about this

aspect has been evident in articles even in the lay press. The article by W. Raab (*Arch. Int. Med.* **101,** *194 (1958)*) touches on many of the human studies and emphasizes the possible influence of our sedentary life on the extent of vascular disease.

The many variables characteristic of human studies make assessment of the influence of individual factors difficult, while studies using experimental animals permit the control of many variables (*Nutrition Reviews* **16,** *274 (1958)*). Even so, not all results of animal experimentation in this area are consistent. Accordingly, S. D. Kobernick and Gen Niwayama (*Am. J. Path.* **36,** *393 (1960)*) have studied further the influence of physical activity on atherosclerosis in rabbits.

New Zealand white rabbit litter mates of both sexes were divided into a sedentary and an exercised group and fed a stock diet mixed with cholesterol. The daily cholesterol intake was 0.75 g. in Experiment I, which lasted 58 days. In Experiment II the intake was 0.5 g. during the first week and 0.25 g. during the remaining 76 days. In the third experiment, lasting 189 days, the daily intake was 0.15 g.

Exercising was achieved either by careful manipulation of a manually operated drum (Experiment I) or by use of an electrified drum containing an electrostimulator (Experiments II and III). Exercising lasted 20 minutes per day in Experiment I and ten minutes per day seven days per week in Experiment II. In Experiment III exercise lasted ten minutes per day, six days per week.

Blood samples were collected at two week intervals and analyzed for free and total cholesterol, phospholipid and total lipid. At the end of the experiment aortas were collected, fixed, graded for lesion severity, sampled for histology then extracted for lipid chemistry.

All cholesterol-fed animals developed atherosclerosis. In Experiment I using the manually operated drum, six of the nine sedentary animals showed relatively severe atherosclerosis (two–three plus) and the severity was less in exercised animals (one plus). Only four of 18 exercised animals in Experiment II had greater than one plus atherosclerosis compared with 15 of 18 non-exercised animals having lesions graded two plus to three plus. Similarly, in Experiment III only one exercised rabbit showed aortic atherosclerosis exceeding one plus. Eleven of 17 sedentary controls, however, showed lesions graded two plus to four plus. The electrified treadmill appeared to be superior in reduction of atherosclerosis.

The weights of the aortas of 17 sedentary animals averaged 831 mg. Total cholesterol was highest in those aortas with an obvious high grade of atherosclerosis ranging from 1.5 mg. to 24.1 mg. and averaging 8.1 mg. or about 1 per cent of the aorta weight. Free cholesterol averaged about 4.5 mg. or a little more than half the total. Phospholipid was variable, ranging from about 0.1 mg. to 1.6 mg. and averaging 0.48 mg. or about 0.6 per cent of aorta weight.

Total cholesterol was elevated in only one of 15 exercised animals which also had the largest free cholesterol, phospholipid and weight of aorta as well as severity of atherosclerosis grossly. The average of all aortas from the exercised animals was 794 mg. Total cholesterol averaged about 3.32 mg. or a little more than 0.4 per cent of aorta. This is about 40 per cent as much as the total cholesterol recovered from the aortas of sedentary animals. Free aortic cholesterol in the exercised rabbits averaged 1.9 mg. which was a little more than half the total of the controls. Phospholipid values showed more variation but averaged about the same in both groups.

Average prefeeding serum total lipids ranged from 414 to 494 mg. per 100 ml. Total cholesterol ranged from 74 to 113 mg. per 100 ml. and free cholesterol ranged from 16 to 50 mg. per 100 ml. Lipid phosphorus averages were between 4.4 and 5.6 mg. per 100 ml. During cholesterol feeding the total

lipids increased in all animals in proportion to the amount of daily cholesterol feeding.

Comparison of average serum lipids, including free and total cholestesterol and phospholipid of exercised and sedentary animals, showed no statistically significant differences. Three animals from Experiment III (receiving 0.15 mg. of cholesterol daily for 189 days) were compared as to the severity of the grade of atherosclerosis and the level of serum total lipid, total and free cholesterol and lipid phosphorus. The animal with the highest levels of serum lipids had the least atherosclerosis. The animal with the lowest levels of serum lipids had an intermediate grade of severity of atherosclerotic lesions and the third animal with serum lipid levels between the other two had the most severe lesions. Similar lack of correlation was characteristic of all other litters.

Although the mechanically induced exercise was not adequate for reducing the extent of atherosclerosis in Experiment I, the amount of cholesterol fed was large and may have masked an exercise effect. The sedentary animals in Experiment II showed more atherosclerosis than in Experiment III although the exercise was comparable in both groups. The amount of cholesterol given was the same but resulted in more deposition in Experiment II in which it was given in a shorter time.

Homogeneity of experimental groups was improved by using litter mates. The effect of exercise was apparent within the litters as well as in whole groups but individual animal variation was typical.

The lack of correlation of serum lipid values and atherosclerosis was puzzling in the light of the findings of others. The atherosclerosis appeared to reflect the amount of cholesterol given and the length of time, indicating that the metabolic turnover of the serum lipids was more significant than the absolute level. The authors propose that metabolic balance studies as well as histochemical analysis of the aorta would be valuable further extensions of their experiments.

HYPERTENSION AND SALT AVERSION

Rats made hypertensive by encapsulation of their kidneys show an aversion to such salt solutions as sodium chloride, sodium sulfate and potassium chloride.

Evidence points to a relation between the development of hypertension and a high salt intake. This evidence is based partly on the demonstration that when rats are maintained on a good diet containing all the necessary ingredients, they show an elevated blood pressure if the diet contains a high level of sodium chloride (*Nutrition Reviews* **15,** *284 (1957)*). An increasing amount of data is appearing which suggests a relation between the quantity of salt ingested by human beings and the development of hypertension (*Nutrition Reviews* **18,** *97 (1960)*). It has been recognized for some time that when hypertensive patients are put on a low sodium diet, the blood pressure falls and remains low as long as the sodium restricted regimen is followed (*Nutrition Reviews* **7,** *257 (1949)*; **10,** *5 (1952)*).

The above observations suggested to M. J. Fregly that it might be advisable to investigate "preference" of the hypertensive rat for sodium chloride. He produced hypertension in his animals by encapsulating their kidneys in molded latex envelopes (*Am. J. Physiol.* **182,** *139 (1955)*). When Sprague-Dawley rats weighing 250 g. were so treated, their systolic blood pressure increased rapidly over a four-week period from a control value of 130 mm. of mercury to 180 mm. During the next 14 weeks the blood pressure increased more slowly to 190–200 mm. where it remained for the subsequent 12 weeks of the study. Control (non-operated) and sham-operated rats (kidneys manipulated as in

regular operation but latex mold sewed between muscle layers of the back) showed increases in systolic blood pressure which averaged only 10 mm. for the entire 30-week period.

One week post-operative, 12 operated, six control and three sham-operated rats were put into metabolism cages to which were attached two bottles, one containing distilled water, the other a solution of 0.15 molar sodium chloride. The position of the bottles was changed daily to avoid habit formation. Ground Purina laboratory chow was fed to all rats.

At the start of the metabolic period, both the control and sham-operated rats showed a reduction in the consumption of distilled water. At the start of the experiment, these rats drank 7 ml. per 100 g. of body weight per day which went down to 4 ml. after three weeks. The rats with the encapsulated kidneys showed no change in water intake for the first three weeks, but then increased their water intake to 11 ml. per 100 g. of body weight by the tenth week of the experiment. It remained at this value with only minor variations for the remainder of the study.

The daily consumption of the 0.15 molar saline solution decreased for the control and sham-operated rats from an initial value of 9 ml. per 100 g. of body weight to 6 ml. by the twelfth week of the experiment where it stayed for the remainder of the study. The hypertensive rats decreased their intake of saline to 4 ml. by the seventh week and then gradually reduced it to 2 ml. by the thirtieth week.

The food intake was the same for all three groups. During the first half of the experiment, the body weights for all three groups increased from the initial value of 250 g. to 400 g. The hypertensive rats showed no further change in body weight, while the other two groups gradually increased in weight to 450 g.

Seven of the 12 hypertensive rats died during the course of the experiment with the first death occurring in the ninth week. Five of these seven rats showed some degree of kidney hypertrophy with a slight amount of mesenteric periarteritis. None of the six control or three sham-operated rats died during the experiment.

The above experiment suggested that rats made hypertensive by encapsulation of the kidneys develop an aversion for sodium chloride solutions which becomes prominent about the time the blood pressure reaches elevated levels.

In an extension of the above work, Fregly (*Am. J. Physiol.* **187,** *288 (1956)*) used the same operative procedure to produce hypertension in rats weighing 410 to 440 g. Since the sham-operated rats in the previous study behaved the same as the non-operated controls, only the latter were used in this work. The concentration of the sodium chloride solution offered the rats was increased every fourth day from an initial value of 0.001 molar to 0.105 molar in 12 stages. As the concentration of the saline solution was increased, the volume of the solution consumed by the control rats remained fairly constant at 5 ml. per 100 g. of body weight per day through the 0.032 molar solution. Concentrations above the latter produced a gradual increase in intake to 7.5 ml. The total fluid intake, however, remained constant throughout the experiment.

The hypertensive rats consumed 9 ml. of saline solution and maintained this level until the sodium chloride concentration reached 0.008 molar. At that time, there was a gradual increase in volume to 15 ml. at 0.060 molar. As the concentration of salt was increased above that, the volume consumed decreased to about 7 ml. The volume of distilled water consumed by the hypertensive rats increased gradually from about 6 ml. to 8 ml. when the rats received the 0.060 molar saline solution. At that point, the water intake increased abruptly to about 12 ml. for the highest saline concentrations.

Fregly used the preference threshold as a means of evaluating the ability of the rats to discriminate between distilled water and the saline solutions. The preference threshold was developed by C. P. Richter (*Endocrinology* **24,** *367 (1939)*) who defined it as the concentration of the saline solution at and above which the simultaneous volumes taken from test and reference bottles differ from each other ($P < .05$). The preference threshold for both control and hypertensive rats lay between 0.008 and 0.016 molar. The similarity in the preference thresholds suggests that the salt aversion of the hypertensive rats is not due to an increased ability to differentiate between saline solutions and water. No explanation could be given for the salt aversion of the hypertensive rats offered saline solutions more concentrated than 0.090 molar.

The food intake of the two groups of rats was essentially the same throughout the experiment. Since the diet contained 221 mEq of sodium per kg., it provided the major share of the sodium intake as long as the concentration of the saline solution was below 0.008 molar. Above this concentration, the saline solutions provided a significant fraction of the total sodium intake. For the hypertensive rats, the maximum sodium intake of 20 mEq per 100 g. of body weight occurred at a saline concentration of 0.045 molar. A similar intake appeared in the control rats only when the saline concentration approached 0.105 molar.

To see whether the sodium chloride aversion of hypertensive rats was a general reaction to all salt solutions, Fregly (*Am. J. Physiol.* **196,** *1326 (1959)*) determined the preference and aversion thresholds of control and hypertensive rats for a variety of salts. During the first week, all animals were offered distilled water in both bottles. Only those rats which drank approximately the same volume of fluid from each bottle were used in the subsequent studies. For four-day periods, five hypertensive rats were offered one of nine potassium chloride solutions ranging in concentration from 0.005 to 0.100 molar. The control rats ingested significantly more potassium chloride solution than water at all concentrations above 0.105 molar. The hypertensive rats ingested more saline than water only at potassium chloride concentrations of 0.010 and 0.015 molar; at concentrations above these, the volume of water ingested by the hypertensive rats was much greater than that of saline.

A similar experiment with sodium sulfate solutions of the same concentrations again showed preferential intake of the saline solution by the control rats and a discrimination against the saline by the hypertensive rats.

For all salt solutions tested, the control and hypertensive rats had the same preference thresholds. Even though the saline concentration that could be detected by the hypertensive rats was the same as that for the normotensive controls, the former reduced their intake of saline solution when the concentration was at or above the threshold, while the controls increased their intake. The increased saline intake was maintained by the controls until the salt concentration was three to ten times greater than the preference thresholds. The volume of saline consumed by the control rats was greater than the intake of distilled water, which decreased as the intake of saline increased. On the other hand, the hypertensive rats increased their consumption of water as their intake of saline was reduced. At no time did the volume of the saline solution ingested by the hypertensive rats exceed the water intake.

One can only speculate as to the physiological advantage accruing to the hypertensive rat from its aversion to salt solutions. It may revolve around an ameliorative effect which is suggested by Fregly's statement (*Am. J. Physiol.* **196,** *1326 (1959)*) that forcing the hypertensive rat to ingest sodium chloride by incorporating it in the feed results in an increase in blood pressure, decreased growth and development of ascites.

MECHANISM OF THE THIAMINE-SPARING ACTION OF DIETARY PENICILLIN IN RATS

Dietary pencillin exerts a thiamine-sparing action in rats by increasing the intestinal synthesis of this vitamin without affecting its absorption or tissue utilization.

Supplementation of B vitamin-deficient diets with any of several antibiotics has been observed to exert a sparing action on the B vitamins (*Nutrition Reviews* **10,** *107 (1952)*). The mechanism of action of the antibiotics in this regard is still largely unknown. Several recent studies have reported that there is increased intestinal synthesis (*Ibid.* **12,** *166 (1954)*), decreased absorption or utilization by intestinal bacteria (*Ibid.* **14,** *124 (1956)*), increase in the absorption by the gut (N. B. Guerrant and J. M. Steel, *Proc. Soc. Exp. Biol. Med.* **98,** *542 (1958)*), or increase in the available vitamin supplied by the intestinal microorganisms (M. S. Mameesh and B. C. Johnson, *J. Nutrition* **65,** *161 (1958)*). In a recent study the mechanism of thiamine-sparing action by dietary penicillin is further explored by a series of well-executed experiments (R. H. Barnes, E. Kwong, K. Delany, and G. Fiala, *J. Nutrition* **71,** *149 (1960)*).

In this study, male weanling rats of the Holtzman strain were housed individually in wire-bottomed cages in a temperature controlled room. For three days, they were fed a completely purified diet containing all known essential nutrients in adequate amounts and then separated into groups. Thiamine was removed from the B-vitamin mixture in the diet for the thiamine-deficient group and 50 mg. of crystalline procaine penicillin per kilogram diet was added to that of the penicillin-supplemented groups. For thiamine determinations, fresh feces were extracted immediately with 0.1 N HCl and the extracts stored at 4°C. until ready for analysis. Total thiamine was determined by the method of D. J. Hennessey and L. R. Cerecedo (*J. Am. Chem. Soc.* **61,** *179 (1939)*).

Several separate experiments were carried out in order to reveal the action of penicillin. The first experiment was designed to study the effect of coprophagy on thiamine-sparing by penicillin. Twelve groups of rats were formed. Four groups received a complete, thiamine-supplemented diet; four a diet containing a low level (0.25 mg./kg.) of thiamine; and four a thiamine-free diet. Two of each of these groups received penicillin in the diet and coprophagy was prevented in one group receiving penicillin and in one group without it for each level of thiamine used. Over a four-week period growth was noted in rats allowed to eat their feces in both the group fed a thiamine-free diet and in the group fed the low thiamine diet. Prevention of coprophagy caused the rats fed the two thiamine-deficient diets to lose weight. Penicillin supplementation in rats allowed access to their feces resulted in accelerated growth in the thiamine-deficient groups. Thiamine-sparing was dependent on coprophagy because prevention of fecal access resulted in marked loss of weight.

In a second experiment designed to study the growth response to graded doses of thiamine with or without penicillin in the diet, ten groups of six rats each were fed a thiamine-free diet and coprophagy was prevented in all groups. The diets of five groups were supplemented with penicillin and five groups were fed the diet without the antibiotic. A water solution of thiamine hydrochloride was administered daily, six times per week by stomach tube so that both penicillin-supplemented and penicillin-free groups received either 0, 2, 4, 6 or 8 μg. thiamine hydrochloride each day. Penicillin did not affect the growth response to a given dose of thiamine during a four-week period. Since prevention of coprophagy might depress the fecal flora, the previous experiment was repeated in modified form in order to determine whether the action of penicillin was dependent on the presence of bacteria in the upper gastrointestinal tract. Copro-

phagy was prevented by fecal collection cups. Feces collected from another set of rats on a low-thiamine, glucose-containing diet were fed to the experimental animals. Although these fecal supplements included a low level of thiamine, in addition to that given by stomach tube, penicillin still did not affect the growth response of these animals to a particular level of thiamine even when the upper gastrointestinal tract was constantly reinoculated with microorganisms. These experiments were interpreted as showing that penicillin affects neither the absorption of thiamine from the upper gastrointestinal tract nor the tissue requirements for this vitamin.

In a third series of experiments the effect of dietary penicillin on the amount of thiamine in the feces was studied. Six groups of ten rats each were fed a thiamine-free diet for four weeks. Three of the groups received a penicillin supplement. Thiamine hydrochloride was given daily by stomach tube at levels of 0, 4 and 8 μg./rat/day for six days to groups fed penicillin-supplemented or penicillin-free diets. Coprophagy was prevented in all animals. Feces were collected for seven days during the third week and the collection for each day was pooled for the rats in a given group and the thiamine content was analyzed. The rats were killed at the end of four weeks and the livers from each group were pooled for thiamine analysis. Penicillin increased intestinal synthesis as indicated by increases of total fecal output of thiamine from 25 to 60 per cent in animals supplemented with penicillin. This increase represented an increase in concentration of thiamine in the feces and was not due to increased amount of feces.

A fourth experiment was designed to determine the proportion of fecal thiamine available to the rat and the effect of penicillin on the amount of available thiamine. This was done in order to determine if the increase in fecal thiamine in penicillin-supplemented rats is great enough to account for the increased growth rate observed. Two groups of ten rats each were fed the thiamine-free diet for four weeks. Coprophagy was prevented in all rats. In one group not fed penicillin, feces were collected in tail cups and fed to the same rats. A second group, fed penicillin, was similarly treated. Body weights were recorded for all animals weekly for four weeks. The amount of feces consumed by each rat was determined by weighing the amount offered and the amount left at the end of the day and the percentage of fecal output eaten was calculated. Total fecal thiamine determinations were made on a one-day sample from each rat. At the same time, four groups of ten rats each were fed a thiamine-free, penicillin-free diet and given daily, six days a week either 0, 2, 4, or 8 μg. thiamine hydrochloride/rat/day by stomach tube. Weight gain at the end of the third week was plotted against the log of the average daily dose of thiamine. From these data the thiamine required in order to account for the three-weeks' growth in the rats fed feces was determined. From the average fecal output, the percentage of fecal ingestion and the total fecal thiamine determinations, the total fecal thiamine consumed each day was calculated. Growth curves provided an estimate of the "available" thiamine consumed on an average for each day. By comparing the total ingested thiamine in the feces with the total available, the percentage of "available" thiamine for each of the different groups was estimated. Approximately 35 per cent of the fecal thiamine was in the available form. Dietary penicillin did not influence the availability of fecal thiamine.

From this series of masterfully planned and executed experiments the authors conclude that intestinal microflora are necessary for the vitamin-sparing action of antibiotics, already noted in germ-free animals (B. S. Wostmann, P. L. Knight and J. A. Reyniers, *J. Am. Chem. Soc.* **66,** *577 (1958)*). They further conclude that thiamine is not absorbed from the lower gastrointestinal tract and that coprophagy is necessary for the utilization of thiamine synthesized

within the intestine. From these clear-cut results it can be inferred that the thiamine-sparing action of penicillin can be entirely accounted for by increased intestinal synthesis of thiamine. This is probably brought about by changes in the microflora since this antibiotic alters neither the tissue requirement, intestinal absorption, nor the proportion of fecal thiamine available to the organism.

ESSENTIAL FATTY ACID ACTIVITY OF TRANS ACIDS

Cis, trans and trans, trans-linoleic acids have neither essential fatty acid (EFA) activity nor anti-EFA activity when fed to weanling rats at 50–100 mg. per day.

The possibility that the trans isomers of the essential fatty acids might have an action antagonistic to that of the essential fatty acids themselves has been of considerable interest because of the formation of such isomers during the partial hydrogenation of vegetable oils, as in the manufacture of margarines (*Nutrition Reviews* **16,** *59 (1958)*). However, the results of experiments to determine whether such is the case have been equivocal or in conflict.

Although it was shown in several laboratories that the conjugated and trans acids do not possess EFA activity, R. T. Holman and E. Aaes-Jorgensen (*Proc. Soc. Exp. Biol. Med.* **93,** *175 (1956)*) interpreted the results of their experiments as indicating that trans acids possess "anti-EFA" activity, while R. B. Alfin-Slater and co-workers (*Proc. Soc. Exp. Biol. Med.* **95,** *521 (1957)*) found that the trans octadecenoic acids, at least, do not interfere with the utilization of essential fatty acids. Since the Committee on Fats in Human Nutrition of the National Research Council (1958) suggested on the basis of the study of Holman and Aaes-Jorgensen that the trans isomers of linoleic acid might hasten the development of EFA-deficiency symptoms under certain conditions, there was evidently need for a definitive answer to this question.

F. H. Mattson has reported the results of experiments in which trans acids of proven identity and purity were tested on adequate numbers of experimental animals (*J. Nutrition* **71,** *366 (1960)*). Weanling male rats that had been on a standard fat-free diet until cessation of weight gain were divided into groups of ten and fed the same diet supplemented with 50 or 100 mg. per day of cis, trans- or trans, trans-linoleate, oleate or elaidate either alone or in combination with an equal amount of cis, cis linoleate. At the end of four weeks, weight gains of the various groups were compared. When supplementary cis, cis linoleate was fed, the weight gain was significantly greater than for the fat-deficient controls. This was true whether it was given alone or combined with any of the other acids. However, when cis, cis linoleate was omitted, none of the trans acids or oleate produced weight gains significantly different from those of the fat-free group.

It was apparent from these results that under the conditions of the experiment, the trans acids had neither EFA activity nor "anti-EFA" activity. The suggestion was made that the discrepant results of Holman and Aaes-Jorgensen might be the result of the small numbers of animals used in experiments which give typically wide spreads of data.

These experiments seem theoretically logical as the trans isomers do not have the same shape as cis acids but resemble the corresponding saturated acids, which do not have anti-EFA activity. Thus, there is no theoretical basis for a belief that either the trans or conjugated acids would have such activity. However, it is possible that the present experiments did not actually test the hypothesis. Although optimal EFA action in rats may be given by 100 mg. per day of linoleic acid, it would be surprising

if this amount of antagonist would be sufficient. It should be of interest to repeat these experiments with considerably larger amounts of the trans acids.

NOTES

Letter to the Editor

Dear Sir:

May I object to some of the statements in the review of vitamin E therapy in your August 1960 issue?

"Uncontrolled" reports on consecutive unselected cases treated by one man are the rule rather than the exception. If your reviewer will look at issues of the great medical journals he will realize this.

Furthermore, there are objections to controlled studies upon private patients on legal, ethical and moral grounds. These have been pointed out in recent articles in the *J.A.M.A.* **167,** *2190 (1958)*; **169,** *181 (1959)*.

My paper did define its terms. I then went to considerable pains to indicate the data provided by comparable ("unworthy"?) series of repute. No exact dose could be stated, although this feature was discussed explicitly. This would have been as true if I had administered insulin or digitalis. My patients were not a Wistar strain, standarized for colour and weight.

You point out that "others had failed to confirm my claims" on vitamin E "therapy" in pre-eclampsia, pruritus vulvae, angina and coronary disease, infertility, threatened abortion and nephritis. This was less than accurate, for some 300 groups of authors have supported these views. May I refer to four recent references which could change this opinion? P. D. Livingstone and C. Jones (*Lancet* **II**, *602 (1958)*); S. Tolgyes and E. Shute (*Canad. Med. Assn. J.* **76,** *730 (1957)*); H. Kawahara (*Surgery* **46,** *768 (1959)*); Nagoya (*J. Med. Sci.* **22,** *341 (1960)*).

I object to details of this last statement itself. I have always strongly urged that vitamin E should *not* be used in pre-eclampsia. To be specific, 17 papers in the literature support our use of alpha tocopherol in nephritis, nine its use in pruritus vulvae; the 11 cited in the paper in question support its use in threatened abortion, at least nine its use in infertility and scores support its use for angina and coronary disease.

I feel that your reviewer should have been more accurate. We, like many others, have long contended for the use of certain chemical substances (which also happen to be "vitamins") as specific pharmacodynamic agents. One uses them just as one would use any other drug to do specific things because they possess specific powers. In my study on abortion I was obviously using alpha tocopherol principally for its role as a vasodilator (of placental vessels) and as an oxygen conserving agent.

Evan V. Shute, F.R.C.S. (C)
The Shute Institute
London, Ontario, Canada

Methoxychlor—Not a Milk Residue Problem

Circumstances resulting in the establishment of a zero tolerance for pesticides in milk has led to a serious re-evaluation of materials used in controlling insects injurious to dairy cattle. The chlorinated hydrocarbons generally used as insecticides have shown a wide variation in the levels required for effectiveness in insect control as well as in the amounts which might be transferred to tissues and to milk (*Nutrition Reviews* **8,** *235 (1960)*).

Of the chlorinated hydrocarbon group, one of the most effective as a topically applied insecticide has been methoxychlor. R. H. Carter *et al.* (*J. Econ. Entomol.* **42,** *116 (1949)*) made a careful study of the excretion of methoxychlor in milk of dairy cattle subjected to various topical applications. They were unable to detect the presence of the insecticide in the milk when a wettable powder containing 0.5 per cent methoxychlor

was used. The method used was sensitive to a few tenths of a part per million.

W. I. Patterson and A. J. Lehman (*Assn. Food and Drug Officials U. S., Quart. Bull.* **17,** *8 (1953)*) established that there is a very low retention of methoxychlor in the body tissues indicating the safety of this pesticide for use on dairy cattle.

Since the widespread use of methoxychlor other investigators have reported that despite different application methods methoxychlor may still be detected in milk. H. V. Claborn and R. W. Wells (*Agr. Chem.* **7,** *28 (1952)*) reported that a cow sprayed with a 0.5 per cent emulsion of methoxychlor produced milk containing 0.7 p.p.m. of this compound the next day; but the cow produced milk with a somewhat lower level if sprayed with a wettable powder suspension. Other investigators have reported conflicting results with some reporting less than 0.1 p.p.m. of methoxychlor excreted in milk following topical applications.

M. L. Cluett *et al.* (*J. Agr. Food Chem.* **8,** *277 (1960)*) extensively investigated the transference of methoxychlor into milk following topical application to dairy cattle. They have applied the pesticide in numerous forms and at levels much higher than those which are recommended for insect control. Since the common complaint in identifying pesticide residues in food products has been concerned with method sensitivity, these investigators went to great lengths to use methods that would identify methoxychlor or any of its metabolites which might appear in milk. The limitation of their results was established by the chloride blank of 0.02 p.p.m., a value much lower than those reported by earlier workers.

The results reported by Cluett *et al.* show that samples of milk taken immediately after application of the methoxychlor and daily thereafter for three weeks did not show detectable amounts of the pesticide or any of its metabolites as indicated by the presence of organic chlorides.

In order to establish parameters in the use of the methoxychlor, Cluett *et al.* investigated the effects on milk following treatment with aqueous suspensions and emulsions and the use of the insecticide at levels up to four times those recommended. These investigations detected the presence of methoxychlor in the milk. Following spraying, a maximum level of 0.1 p.p.m. was found the first day after application, but the amount decreased rapidly to levels below detectable amounts within one week following treatment.

When cattle were dusted with four times the amount of powder recommended, methoxychlor appeared in milk at a level of 0.05 p.p.m. shortly after the topical application and rapidly disappeared therefter. When the same high levels were sprayed on as a wettable powder, the level of methoxychlor in milk rose to a high of 0.23 p.p.m. and again rapidly disappeared in succeeding samples. Careful examination was made of milk following repeated applications of the various methoxychlor formulations over an 18-week period with the conclusion that there was no accumulation of this compound in milk.

Comparing analyses for methoxychlor and for organic chlorides which might indicate the presence of possible metabolites, it was apparent that all of the chlorides could be accounted for in terms of the methoxychlor, indicating that secondary compounds are not a problem in the metabolism of this pesticide.

As an effective insecticide, the compound methoxychlor has shown very low mammalian toxicity and good pest control properties. Consequently, the report of Cluett *et al.* is especially valuable since they have combined a careful animal study with analytical methods of exceptional sensitivity. Their results show the absence of the chlorinated hydrocarbon in milk if insecticides are applied as recommended. This is encouraging both to the farmer and to health officials since it suggests the possibility of effectively controlling insects without introducing residues into the food product.

BOARD OF TRUSTEES

W. GARDNER BARKER, *President, Thomas J. Lipton, Inc.*

*CLAUDE T. BISSELL, *President, University of Toronto*

*F. G. BOUDREAU, *President, Milbank Memorial Fund.*

WILLIAM T. BRADY, *Chairman, Corn Products Company.*

CHARLES S. BRIDGES, *President, Libby, McNeill & Libby*

G. BUITONI, *President, Buitoni Foods Corp.*

*LEROY E. BURNEY, *Surgeon General, U. S. Public Health Service*

*CASON J. CALLAWAY, *Hamilton, Georgia*

*O. C. CARMICHAEL, *Biltmore, North Carolina*

THOMAS P. CARNEY, *Vice President, Research, Development & Control, Eli Lilly and Company*

HAROLD W. COMFORT, *President, The Borden Company*

GEORGE H. COFFERS, *Chairman, National Biscuit Company*

HERBERT C. CORNUELLE, *Pres. Dole Corp.*

W. T. CRIGHTON, *General Manager, Producers Creamery Company*

WESLEY M. DIXON, *Chairman, Container Corporation of America*

*LEE A. DUBRIDGE, *President, California Institute of Technology*

JOSEPH EICHBERG, *President, American Lecithin Co., Inc.*

*CONRAD A. ELVEHJEM, *President, University of Wisconsin*

THOMAS C. FOGARTY, *President, Continental Can Co., Inc.*

EDGAR J. FORIO, *Senior Vice President, The Coca-Cola Company*

*CLARENCE FRANCIS, *New York, New York*

T. R. GAMBLE, *President, Pet Milk Company*

DANIEL F. GERBER, *President, Gerber Products Co.; Vice President, The Nutrition Foundation, Inc.*

GEORGE A. GRAHAM, *Secretary Treasurer, Charles B. Knox Gelatine Co., Inc.*

JOHN A. GRAMMER, *Executive Vice Preside[illegible] Beech-Nut Life Savers, I[illegible]*

HENRY J. HEINZ, II, *Chairman, H. J. Heinz Co.*

*REV. THEODORE M. HESBURGH, C.S.C., *President, University of Notre Dame*

FRANK C. HILDEBRAND, *Vice President, General Mills, Inc.*

*HERMAN E. HILLEBOE, *Commissioner of Health, State of New York*

*FREDERICK L. HOVDE, *President, Purdue University*

PORTER M. JARVIS, *President, Swift & Company*

*JAMES R. KILLIAN, JR., *Chairman, Massachusetts Institute of Technology; Chairman of Board of Trustees, The Nutrition Foundation, Inc.*

*CHARLES GLEN KING, *Executive Director, The Nutrition Foundation, Inc.*

*GRAYSON L. KIRK, *President, Columbia University*

R. NEWTON LAUGHLIN, *President, Continental Baking Co.*

J. PRESTON LEVIS, *Chairman, Owens-Illinois*

R. G. LUCKS, *President, California Packing Corp.*

W. F. MCLEAN, *President, Canada Packers, Limited*

WILLIAM H. MCLEAN, *President of Chemical Division, Merck & Co.*

OSCAR MAYER, *Chairman of the Board, Oscar Mayer & Co.*

CHARLES G. MORTIMER, *Chairman, General Foods Corporation: President, The Nutrition Foundation, Inc.*

MILTON C. MUMFORD, *President, Lever Brothers Co.*

W. B. MURPHY, *President, Campbell Soup Co.*

H. EARLE MUZZY, *Vice Chairman, The Quaker Oats Co.*

WILLIAM F. OLIVER, *President, The American Sugar Refining Company*

W. W. PADDON, *President, Sunshine Biscuits, Inc.*

*THOMAS PARRAN, *President, Avalon Foundation*

*MAURICE PATE, *Executive Director, United Nations Childrens Fund*

PHILIP W. PILLSBURY, *Chairman, The Pillsbury Company*

J. G. PLEASANTS, *Vice-President in charge of Research and Development, The Procter & Gamble Company*

B. E. RICHMOND, *President, Richmond-Chase Company*

LLOYD E. RIGLER, *President, Adolph's, Ltd.*

LYLE C. ROLL, *President, Kellogg Company*

R. T. RYAN, *Executive Vice President, Welch Grape Juice Co., Inc.*

ELBERT M. SHELTON, *Secretary, The Baker Laboratories Inc.*

VERNON STOUFFER, *President, The Stouffer Corporation*

ROY J. SUND, *President, American Can Company*

HENRY WEIGL, *President, Standard Brands, Incorporated*

J. HUBER WETENHALL, *President, National Dairy Products Corporation*

F. R. WILCOX, *General Manager, Sunkist Growers*

E. W. WILSON, *Executive Vice President, Armour & Company*

R. D. L. WIRTH, *President, Red Star Yeast and Products Co.*

H. J. WOLFLISBERG, *President, The Nestle Company, Inc.*

HARRY W. ZINSMASTER, *Chairman, Zinsmaster Baking Co.*

HORACE L. SIPPLE, *Secretary* JOHN HOLMES, *Honorary Trustee*

---

* Representative of the public.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.